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585895

Brokd 1800

PATENT APPLICATION



08585895

CONTENTS *pgs*

Date Entered or Counted

APPROVED FOR LICENSE

INITIALS \_\_\_\_\_

Date Received or Mailed

1.	Application	24 Sheets papers	
2.	Raw Request Picting (OK)		8/16/96
3.	Exh. B. Declaration of Invention		APR 14 1996
4.	Petition (S3)		5/24/96
5.	Petition granted (G3)		7/22/96
6.	Best. 30 days		11/25/96
7.	Pre Amt 1A		8-14-96
8.	I.O.S. v. Attch		11-1-96
9.	I.D.S.		12-23-96
10.	PETITION TO EXPEDITE (PETITION)		06-25-96
11.	I.D.S.		02-11-97
12.	I.D.S.		03-24-97
13.	I.D.S.		7/23/97
14.	Exh. B. Declaration of Invention		7/27/97
15.	Declaration of Invention		1/27/97
16.	Info. Discl. Stmt		4/21/97
17.	RES 3 mos		5-28-97
18.	Ext. of Time (3 months)		12/6/97
19.	Amend. Declaration / CF Doc		12/6/97
20.	Power of Attorney		02/26/98
21.	Raw Request Picting		01/22/98
22.	Res 3 mos		13-24-98
23.	Declaration		7/23/98
24.	Ext. of Time 1 month		7-27-98
25.	Sec 1		7-27-98
26.	Amend. - 1		7-27-98
27.	Letter of Suspension		10/8/98
28.	STID		7-28-99
29.	S.I.D.S.		Oct. 28, 1999
30.	Req. 3 months		4/10/00
31.	Power of Attorney		10-22-00
32.	Interview Summary		6-28-00

Amend. Inside (FRONT)

Class 435 Subject 69.4 ISSUE CLASSIFICATION		UTILITY SERIAL NUMBER 585895 PATENT DATE JUN 12 2001 PATENT NUMBER 6245530	
SERIAL NUMBER 08/585,895	FILING DATE 01/12/96	CLASS 435	SUBCLASS GROUP ART UNIT EXAMINER

APPLICANT: KARI ALITALO, ESPOO, FINLAND

\*\*CONTINUING DATA\*\*\*\*\*  
 VERIFIED THIS APPLN IS A CONTIN. OF 02/340,011 11/14/94  
 BRL

\*\*FOREIGN/PCT APPLICATE VERIFIED  
 VERIFIED NONE  
 BRL

Foreign priority claimed	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEYS DOCKET NO.
35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no							

Verified and Acknowledged  
 Examiner's Initials  
 MARSHALL OTTOLE GERSTEIN MURRAY  
 6309 SEARS TOWER  
 233 SOUTH WACKER DRIVE  
 CHICAGO IL 60606-8602

RECEPTOR SIGNATURE

U.S. DEPT. OF COMM./PAT. & TM--PTO-436L (Rev.12-94)

04/05/01 Formal Drawings (30 sheets) set 1 01/24/01	
PARTS OF APPLICATION FILED SEPARATELY	
NOTICE OF ALLOWANCE MAILED 10 18 24 96	CLAIMS ALLOWED Total Claims 35 Print Claim 1
ISSUE FEE (W) Amount Due 1940 Date Paid 1-20-91	DRAWING Sheets Drwg. 2430 Figs. Drwg. 2631 Print Fig. Now
Label Area 1	CHRISTINE SAUND PATENT EXAMINER Christine Saund Primary Examiner PREPARED FOR ISSUE
WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 167 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.	

Form PTO-436A (Rev. 8/92)  
 BRL  
 Partial Drawings 1-24/01



POSITION	ID NO.	DATE
CLASSIFIER		
EXAMINER	4/6	2-22-96
TYPIST		4/15/96
VERIFIER		
CORPS CORR.		
SPEC. HAND	509	7-1-96
FILE MAINT.	323	5-14
DRAFTING		

# INDEX OF CLAIMS

Claim	Date
1	1/15/96
2	1/15/96
3	1/15/96
4	1/15/96
5	1/15/96
6	1/15/96
7	1/15/96
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SYMBOLS

✓ Rejected  
 - Allowed  
 (Through numbers) Cancelled  
 N Restricted  
 A Non-elected  
 O Interference  
 O Appeal  
 O Objected

Claim	Date
1	1/15/96
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1. Application _____ papers.		Date received (incl. C. of M.) or Date Mailed	Date received (incl. C. of M.) or Date Mailed
2.			42.
3.	Phot. line (1) month	Aug. 10/2000	43.
34.	Declaration	Aug. 10/2000	44.
35.	Amendment E	Aug. 10/2000	45.
36.	Declaration	8/14/00	46.
37.	Amendment G	10-24-00 19/17	47.
38.	Amendment G	01/24/01	48.
39.	PTOL 271	02/24/01	49.
40.	Drawings	1/24/01	50.
41.			51.
42.			52.
43.			53.
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72.			82.

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08 58589

PATENT  
28113/33072

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: )  
Alitalo et al. )  
Serial No.: Not yet assigned )  
Filed: Herewith )  
For: Receptor Ligand )  
Group Art Unit: Not yet assigned )  
Examiner: Not yet assigned )

) "EXPRESS MAIL"  
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) and is addressed to the Assistant  
) Commissioner for Patents,  
) Washington, D.C., 20231.  
) David A. Gass  
) David A. Gass

## STATEMENT PURSUANT TO 37 C.F.R. §1.821(f)

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I hereby state that the content of the paper and computer readable forms of the sequence listing that is part of the above-identified application and that are filed herewith are the same.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Dated: January 12, 1996

David A. Gass  
David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, IL 60606-6402  
Telephone: (312) 474-6300

# PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1995

Application or Docket Number

08/585895

## CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	16 minus 20 =	
INDEPENDENT CLAIMS	3 minus 3 =	
MULTIPLE DEPENDENT CLAIM PRESENT		

\* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
	375.00	OR		750.00
x\$11=		OR	x\$22=	
x39=		OR	x78=	
+125=		OR	+250=	
TOTAL		OR	TOTAL	750

## CLAIMS AS AMENDED - PART II

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	23	20	13
Independent	3	3	0
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=	\$143 <sup>00</sup>	OR	x\$22=	
x39=	\$82 <sup>00</sup>	OR	x78=	
+125=	\$135 <sup>00</sup>	OR	+250=	
TOTAL ADDIT. FEE	\$360 <sup>00</sup>	OR	TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total		37	
Independent		5	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=		OR	x\$22=	
x39=		OR	x78=	
+125=		OR	+250=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	35	37	
Independent	8	5	2
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
x\$11=	78	OR	x\$22=	
x39=	78	OR	x78=	
+125=	C	OR	+250=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

\* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.  
 \*\* If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."  
 \*\*\* If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."  
 The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.



US006245530B1

(12) **United States Patent**  
Alitalo et al.

(10) Patent No.: **US 6,245,530 B1**  
(45) Date of Patent: **Jun. 12, 2001**

(54) **RECEPTOR LIGAND**

(75) Inventors: Kari Alitalo, Espoo (FI); Vladimir Joukov, Boston, MA (US)

(73) Assignees: Ludwig Institute for Cancer Research, New York, NY (US); Helsinki University Licensing, Ltd. OY, Helsinki (FI)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 08/585,895

(22) Filed: Jan. 12, 1996

**Related U.S. Application Data**

(63) Continuation-in-part of application No. 08/510,133, filed on Aug. 1, 1995.

(51) Int. Cl.<sup>7</sup> C12N 15/12; C12N 15/63; C12N 5/10; C12N 5/16

(52) U.S. Cl. 435/69.4; 435/70.1; 435/325; 435/320.1; 536/23.51; 530/399; 935/13

(58) Field of Search 536/23.51; 435/252.3; 435/254.11, 320.1, 419, 69.4, 70.1, 325; 530/399; 935/13

(56) **References Cited****U.S. PATENT DOCUMENTS**

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5,332,671 7/1994 Ferrara et al. 435/240.1  
5,607,918 3/1997 Eriksson et al. 514/12  
5,932,540 8/1999 Jing-Shan Hu et al.  
5,935,820 8/1999 Jing-Shan Hu et al.

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WO 96/20046 10/1996 (WO).  
WO 96/39421 12/1996 (WO).  
WO 96/39515 12/1996 (WO).  
97/05250 2/1997 (WO).  
97/09427 3/1997 (WO).  
97/17442 5/1997 (WO).

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Reeck et al. Cell 50, 667, 1987.\*  
Hillier et al. y185b08.21 *Homo sapiens* cDNA clone 44993 5' EST-STS Accession No. H05177, Jun. 21, 1995.\*  
Hillier et al. y186p06.r1 *Homo sapiens* cDNA clone 45138 5' EST-STS Accession No. H07991, Jun. 23, 1995.\*  
Hillier et al. yd29f07.r1 *Homo sapiens* cDNA clone 109669 5' similar to SP.BAR3\_CHITE Q03376 Balbiani Ring Protein 3. EST-STS Accession No. T81690, Mar. 15, 1995.\*  
Aufray et al. *H. sapiens* partial cDNA sequence: clone c-1wf11. EST-STS Accession No. Z44272, Nov. 6, 1994.\*

Pajusola. "Cloning and Characterization of a New Endothelial Receptor Tyrosine Kinase Flt4 and Two Novel VEGF-Like Growth Factors VEGF-B and VEGF-C." Academic Dissertation, Molecular/Cancer Biology Laboratory and Department of Pathology, Haartman Institute and Department of Biosciences, Division of Genetics, University of Helsinki, (Jan. 26, 1996).

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Alitalo et al. "Vascular Endothelial Growth Factors B and C and Receptors Involved in Angiogenesis." *German-American Academic Council Foundation (GAACF) Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease*, Nov. 17-20, 1996, Ringberg Castle, Germany, p. 1 (Abstract).

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Provisional application No. 60/003,491, James Lee and William Wood, Sep. 8, 1995.

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Achen, M.G. et al. "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGFR Receptor 2 (Flk1) and VEGFR Receptor 3 (Flt4)." *Proceedings of the National Academy of Science, USA*, 95:548-553 (Jan., 1998).

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(List continued on next page.)

Primary Examiner—Christine Saoud

(74) Attorney, Agent, or Firm—Marshall, O'Toole, Gerstein, Murray & Borun

(57) **ABSTRACT**

Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligands, pharmaceutical compositions and diagnostic reagents.

35 Claims, 30 Drawing Sheets

SEARCHED			
Class	Sub	Date	Exmr.
530	5351	5/25/67	BUL
535	5353	↓	↓
	5354		
	5357		
	5361		
10/20/68	5362	5/15/68	CP
10/20/68	5363	3/22/60	CR
2533	1694	10/24/60	CR
	1701	↓	↓
	325		
530	3201		
530	2351		
985	399		
	13		

SEARCH NOTES		
	Date	Exmr.
SEE ID NOS. 32 and 33, except attached.	11/15/96	BW
USPAT, MEDLINE, WPIOS searched - see attached.	5/25/97	BW
Update - see search notes in file	3/19/98	KP
seq. search SEQ ID NO: 32-33 update	2/8/00	CL
update	3/25/00	CL
	10/20/00	CL

Class	Sub.	Date	Exm.
35	69.45	10/20/60	✓
36	56.45		
37	32.5		
38	62.61		
39	33.5		
40	33.5		
41	5		

7/24/98

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of: ) I hereby certify that this paper is being  
Alitalo et al. ) deposited with the United States Postal  
Serial No.: 08/585,895 ) Service as first class mail, postage  
Filed: January 12, 1996 ) prepaid, in an envelope addressed to:  
Title: RECEPTOR LIGAND ) Assistant Commissioner for Patents  
Art Unit: 1801 ) Washington, D.C. 20231, on this date:  
Examiner: Lathrop, B. ) Dated: Nov. 26, 1997  
David A. Gass  
Registration No. 38,153

DECLARATION OF BIOLOGICAL CULTURE DEPOSIT  
IN COMPLIANCE WITH BUDAPEST TREATY REQUIREMENTS

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, the undersigned, declare that:

1. I am an inventor of the subject matter of the above-identified patent application.

2. The plasmid designated FLT4-L, described in the specification of the above-identified application at pages 28-29 (and elsewhere), was deposited on 24 July 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, under the terms of the Budapest Treaty. This plasmid was assigned ATCC accession number 97231. A copy of the ATCC deposit receipt, confirming viability of the deposit, is attached hereto.

3. With respect to the permanence of the deposit, the ATCC is an official depository in accordance with the Budapest Treaty for the above-deposited material, and I affirm that, should the plasmid identified in paragraph 2 mutate, become non-viable, or be inadvertently destroyed, I will replace it for at least thirty (30) years from the date of the original deposit, or for at least five (5) years from the date of the most recent request for release of a sample, or for the enforceable life of any patent issued on the above-mentioned application, whichever period is longest.

4. With respect to availability of the plasmid identified in paragraph 2, I affirm that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if an enforceable patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent [MPEP §608.01 (p)], and (b) access to the deposit will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

5. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997  
Date

Kari Alitalo  
Kari Alitalo





# American Type Culture Collection

13341 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301) 231-5520 Telex: 894-455 ATCCNORTH • FAX: 301-770-1587

## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

### INTERNATIONAL FORM

#### RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

University of Helsinki  
Attention: Kari Alitalo  
Molecular/Cancer Biology Laboratory  
P.O. Box 21 (Haartmaninkatu 3)  
SF-00014, HELSINKI, FINLAND

Deposited on Behalf of: Kari Alitalo and Vladimir Joukov

Identification Reference by Depositor: ATCC Designation

Plasmid, FLT4-L 97231

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.The deposit was received July 24, 1995 by this International Depository Authority and has been accepted.

#### AT YOUR REQUEST:

☒ We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain and ATCC is instructed by the United States Patent &amp; Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested August 1, 1995. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

  
Andrew L. Bade, Director, Patent DepositoryDate: August 9, 1995

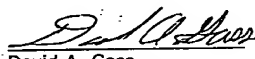
cc: Thomas C. Meyers

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#26  
PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Alitalo *et al.* ) I hereby certify that this paper is  
Serial No. 08/585,895 ) being deposited with the United  
Filed: January 12, 1996 ) States Postal Service as first class  
For: RECEPTOR LIGAND ) mail, postage prepaid, in an  
Art Unit: 1646 ) envelope addressed to: Assistant  
Examiner: Saoud ) Commissioner for Patents,  
 ) Washington, D.C. 20231, on this  
 ) date: July 23, 1998  
 )  
 )   
 ) David A. Gass  
 ) Attorney for Applicants  
 )

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. § 1.111

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

In an official action mailed March 24, 1998, the U.S. Patent and Trademark Office (the Patent Office) allowed claims 33-36, but rejected claims 1, 3-5, 7, 11, 18-32, and 37-38 variously under 35 U.S.C. §§ 112, first and second paragraphs. The Patent Office also objected to an amendment under §132, alleging that the amendment introduced new matter. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This amendment has been timely filed with a petition and fee for one month extension of time, extending the shortened statutory period to July 24, 1998.

## AMENDMENTS

### In the specification:

Please amend the specification as set forth below:

Please delete the amendment to the priority claim at page 1, line 3, filed on August 12, 1996, and substitute therefor the following updated priority claim: <sup>AND</sup> This application is also a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994, now U.S. Patent No. 5,776,755.

At page 5, line 21, delete "SEQ ID NO: 2" and substitute therefor --SEQ ID NO: 33--.

At page 5, line 31, delete "polypeptide" and substitute therefor --polypeptides--.

Please cancel the amendment to page 29, line 1, of the specification made on November 26, 1996, and substitute therefor the following amendment at the same location: --The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a nucleotide sequence that includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The nucleotide sequence set forth in SEQ ID NO: 44 encodes the 419 residue amino acid sequence set forth in SEQ ID NO: 45.

At page 29, line 3, delete "this reading frame" and substitute therefor the reading frame specified in SEQ ID NOs: 32-33.

At page 31, line 20, after "ORF" please insert specified in SEQ ID NOs: 32 and 33.

### In the claims:

Please amend claims 1, 3-5, 7, 18-19, 26-33 and 36-37; and add new claims 39-44 as shown below:

D5

1. (Three times amended) A host cell transformed or transfected with a polynucleotide [encoding a polypeptide that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase], wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32,

under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, [said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)]

wherein said polypeptide includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B)

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase.

D6

3. (Three times amended) A host cell transformed or transfected with a [nucleic acid encoding] polynucleotide comprising a nucleotide sequence that encodes [a polypeptide having] the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, [said polypeptide including a domain defined by eight conserved

cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)) said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC).

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide is capable of binding to Flt4EC.

D6 4. (Twice amended) A host cell according to claim 3 wherein said [nucleic acid] nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

5. (Three times amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising [a nucleic acid] an expression control sequence operatively linked to the nucleotide sequence that encodes [a polypeptide having] the amino acid sequence shown in SEQ ID NO: 33.

D7 7. (Twice amended) A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert. [plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)]

wherein said secreted polypeptide binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human

platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and

wherein said secreted polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P).

18. (Twice amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to [an] human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid [polynucleotide] lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

19. (Amended) A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of human Flt4 receptor tyrosine kinase.

26. (Amended) A host cell according to claim 1 that expresses a naturally occurring [VEGF-C] Flt4 ligand protein encoded by said polynucleotide.

27. (Amended) A host cell according to claim 1 that expresses a human [VEGF-C] Flt4 ligand protein encoded by said polynucleotide.

28. (Amended) A host cell according to claim [27] 1, wherein said host cell expresses said polynucleotide and produces a [mature] human [VEGF-C] protein that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, said protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. (Amended) A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to [a nucleotide] sequence that encodes said polypeptide.

D10 30. (Amended) A [polynucleotide] nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes [a VEGF-homologous portion] eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. (Amended) A [polynucleotide] nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. (Amended) A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence [corresponding] identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. (Amended) A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion



D10 commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

36. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

D11 growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating phosphorylation of Flt4 receptor tyrosine kinase.

37. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), [conserved cysteines and having homology to vascular endothelial growth factor (VEGF)] and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

— 39. A method according to claim 38 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

40. A eukaryotic host cell according to claim 1 or 3 that secretes said polypeptide.

D12 41. A nucleic acid according to claim 30 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

42. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

43. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a

continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

D12 44. A purified and isolated nucleic acid according to claim 43 wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.

#### REMARKS

##### I. History of claims and explanation of amendments.

###### A. Prosecution History

The application as filed contained 16 claims.

In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In an Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In a responsive amendment dated November 26, 1997, the Applicants canceled claims 6, 13, and 17; amended claims 1, 3-5, 7, 11, 18, and 20; and added new claims 26-38. Thus, claims 1, 3-5, 7, 11 and 18-38 were pending at the time the outstanding Office action was issued. In the outstanding Office action, claims 33-36 have been allowed, but claims 1, 3-5, 7, 11, 18-32, and 37-38 were rejected.

In the present amendment, the Applicants amend claims 1, 3-5, 7, 18-19, 26-33, and 36-37; and add new claims 39-44. A copy of the claims, in their amended forms, is appended hereto for the Examiner's convenience.

nucleotides 37 to 1086 represent the portion of SEQ ID NO: 32 that encodes the amino acid sequence specified in SED ID NO: 33.

The amendment to claim 5 to recite a vector comprising "an expression control sequence operatively linked" to a coding sequence finds support throughout the application, including at page 6, lines 28-30.

The amendment to claim 7 to specify that the host cell "secretes" the encoded polypeptide, and to specify that the secreted polypeptide binds to Flt4, is found throughout the application. For example, Example 11 (p. 28) of the application describes the expression and secretion into the cell culture medium of a polypeptide encoded by the insert of the deposited plasmid. The polypeptide bound Flt4 and stimulated Flt4 phosphorylation. New claims 39-40 are likewise supported by way of example (see Examples 6, 11, and 13, for example, teaching the use of eukaryotic/mammalian expression vectors and cell lines to express VEGF-C).

Claims 18' and 19 have been amended to recite "human" (i.e., "human Flt4"). This amendment is not intended to imply that polypeptides of the invention which bind to human Flt4 would not also bind to Flt4 proteins of other animals. Claim 18 also has been amended to recite "nucleic acid" instead of "polynucleotide." This amendment is not intended to alter the scope of the claim, but merely to use a term that has *ipsis verbis* antecedent basis in the preamble.

Claims 26 and 27 have been amended to recite "Flt4 ligand" instead of "VEGF-C." This amendment is not intended to diminish the scope of the claims, since VEGF-C is the name ascribed to an Flt4 ligand of the invention. See, e.g., specification at page 5, lines 17-19. Similarly, claim 28 has been amended to recite a human protein "that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase," instead of reciting human "VEGF-C."

Claim 28 also has been amended to delete the term "mature," which is believed to be unnecessary to define the invention, especially in view of the binding and molecular weight limitations of the claim.

The amendment to allowed claim 33 to recite "said polypeptide *having an amino acid sequence* consisting of a continuous portion of the sequence shown in SEQ ID NO: 33" is formal in nature and not intended to diminish the scope of the claim.

Likewise, the amendment to allowed claim 36 merely makes the language of the final step of the claim more closely parallel the language of the preamble. This amendment is formal in nature and not intended to diminish the scope of the claim.

New claim 41 finds support throughout the application as originally filed, including at page 23, lines 1-10, and claim 10 as originally filed.

New claim 42 is directed to a host cell transformed or transfected with a polynucleotide. The hybridization conditions recited in claim 42 are identical to those recited in claim 1 and find support, e.g., in Example 10 at page 27, lines 10-14. The recitation in claim 42 that the host cell "secretes" the expressed polypeptide finds support, e.g., in Example 11 (p. 28). The size and binding characteristics that are recited in new claim 42 find support throughout the application as originally filed, including in Example 5 and in original claims 8 and 9.

New claims 43-44 find support throughout the application as originally filed, including at page 6, lines 16-20. The specified terminal amino acids in claims 43-44 find support, e.g., at page 5, lines 27-34, and are the same terminal residues specified in allowed claim 33.

- II. The rejection of claims 7 and 37 under 35 U.S.C. §112, first paragraph, was improper, and should be withdrawn.

Paragraphs 4 and 10-12 pertain to a rejection of claims 7 and 37 under 35 U.S.C. §112, first paragraph. The Patent Office indicates that it will withdraw the rejection if an appropriate statement is filed lifting all restrictions on the availability of a deposited plasmid, consistent with Budapest treaty. *The Applicants filed such a statement with their amendment dated November 26,*

1997. A copy of the statement is filed herewith. Thus, the objection to the specification and rejection should now be withdrawn.

III. The objection that a previous amendment introduced new matter should be withdrawn.

In paragraphs 4 and 10 of the Office action, the Patent Office objects to an amendment to introduce SEQ ID NO: 44 and 45 into the application, alleging that the amendment introduces new matter:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97321 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44." It is further noted that the nucleotide sequence of the plasmid is not SEQ ID NO: 45, as stated in the added sentence. SEQ ID NO: 45 is a translated open reading frame of the nucleotide sequence of SEQ ID NO: 44 . . . . .

(Office action at p. 4; see also p. 2.)

The Applicants respectfully traverse.

The allegation that the previously-filed Rule 132 Alitalo declaration fails to state that the cDNA insert was derived from ATCC Deposit No. 97231 is incorrect. Paragraph 4 of the declaration identifies the plasmid by its ATCC accession number and paragraph 5 states, "Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45." Thus, the amendment to add SEQ ID NOs: 44-45 to the application had sufficient corroboration.

Notwithstanding the foregoing, the Applicants have adopted all of the Patent Office's suggestions to overcome the new matter objection. The Applicants have amended the application at page 29 to explain the relationship between the approximately 2.1 kb insert and the 1997 base pair sequence; the Applicants have clarified the DNA/encoded protein relationship between SEQ ID NO: 44 and 45; and the Applicants have filed herewith another declaration from Dr. Alitalo confirming that SEQ ID NOs: 44 and 45 represent nucleotide and deduced amino acid sequences of the deposited plasmid. Accordingly, the new matter objection should now be withdrawn.

IV. The rejection of claims 1, 18, 23-31, and 37-38 under §112, first paragraph, should be withdrawn.

In paragraphs 5 and 13 of the outstanding Office action, the Patent Office rejected claims 1, 18, 23-31, and 37-38 under §112, first paragraph, alleging that the specification does not reasonably enable the full scope of these claims. As its basis for rejection, the Patent Office alleges that neither the application nor the prior art enables one skilled in the art to use a polypeptide which binds to the Flt4 receptor and which does NOT stimulate tyrosine phosphorylation activity of the receptor. (Office action at pp. 5-7.) The Applicants respectfully traverse.

The present patent application teaches uses for polypeptides of the invention that bind, but fail to activate, the Flt4 receptor. For example, at page 7, lines 8-15, the application teaches that Flt4 ligand polypeptides of the invention can be labeled and used to identify their corresponding receptor *in situ*. Such labeled ligands can be used as detection or imaging agents, analogous to anti-Flt4 antibodies, to detect and/or image lymphatic vessels and high endothelial venules that express the Flt4 receptor on their surface. Such imaging/detection uses include uses for analyzing histochemical tissue sections. Those skilled in the art understand that the activity of binding to the extracellular domain of Flt4 is all that is required to make polypeptides effective for such uses. Stated differently, imaging a receptor with a labeled binding

agent does not require the labeled binding agent to activate the receptor. Such uses were discussed in an interview of March 24, 1998, in a related application (USSN 08/671,573), at which time Examiner Brown acknowledged that she had not considered such uses when entering the rejection. A similar rejection in the related application has now been withdrawn by the Patent Office.

The application also teaches that peptides which block the Flt4 receptor are useful as inhibitors to control endothelial cell proliferation and lymphangiomias. (See page 7, line 32, to page 8, line 2.) Persons skilled in the art understand that polypeptides that bind to the receptor but fail to activate the receptor can serve as competitive inhibitors. Thus, the application provides this additional use for polypeptides that bind Flt4 but fail to stimulate tyrosine phosphorylation of the receptor.

Because the present application teaches those skilled in the art "how to use a polypeptide which binds to the Flt4 receptor but which does not stimulate tyrosine phosphorylation," the Patent Office's basis for rejection is unfounded. Accordingly, the rejection of claims 1, 18, 23-31, and 37-38 under § 112, first paragraph, should be withdrawn.

V. The Patent Office's rejections of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. § 112, second paragraph, should be withdrawn.

In paragraphs 15-22 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. § 112, second paragraph, alleging several bases why these claims were indefinite. The Applicants traverse-in-part and amend-in-part.

A. The rejection of claims 1, 3-5, 7, 26-29, and 37 relating to the term "a domain defined by eight conserved cysteine residues" should be withdrawn.

In paragraph 16 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 26-29, and 37, alleging that the term "a domain defined by eight conserved cysteine residues" was indefinite. The Applicants traverse-in-part and amend-in-part.



1. It is clear to what the eight residues are conserved.

As its first rationale for rejection, the Patent Office asserted, "It is unclear to what the eight residues are conserved." (Office action at p. 8.) The Applicants' amendments render this rationale moot. For example, claim 1 has been amended to recite, "wherein said polypeptide includes a domain defined by eight cysteine residues *that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).*" Claims 3, 7, and 37 have been amended similarly. The eight conserved cysteines are readily apparent to scientists skilled in the art. (See the alignment of VEGF, PDGF-A, and PDGF-B in Fig. 10A of the patent application, with conserved cysteines at positions 103, 130, 136, 139, 140, 147, 184, and 186.) Since the claim now recites "to what the eight residues are conserved," the basis for rejection is rendered moot.

2. The minimum limits of the domain are clear.

As its second rationale for rejection, the Patent Office asserted, "It is also unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter." (Office action at p. 8.)

The basis for the rejection is contrary to the plain language of the claims. If a domain is "defined by eight conserved cysteines," then it clearly is not "defined by a different parameter." Thus, the plain language of the claims demonstrates that this basis for rejection is improper, and that the minimum included portion of the encoded polypeptide is defined with particularity. Accordingly, the rejection should be withdrawn.<sup>2</sup>

<sup>2</sup> Moreover, the Applicants' amendments to claim 3 render this basis for rejection moot with respect to claims 3-5, because claim 3 no longer recites "domain defined by." Instead, claim 3 recites a "contiguous portion of SEQ ID NO: 33" that "includes" the eight conserved cysteines.

3. The objection to the term "homology" is now moot.

As a third basis for rejection the Patent Office alleged, "these claims are indefinite with respect to the term 'having homology to vascular endothelial growth factor.' It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF." (Office action at p. 8.)

The Applicants respectfully submit that this phrase is clear and that "similarity" and "common evolutionary origin" are not incompatible concepts. However, solely to expedite allowance, the Applicants have deleted the allegedly indefinite term from claims 1, 3, 7, and 37, rendering this basis for rejection moot.

4. Conclusion

For the reasons set forth above, the rejection of claims 1, 3-5, 7, 26-29, and 37 should be withdrawn.

- B. The rejection of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 with respect to the term "cysteine motifs of a Balbiani ring 3 protein" should be withdrawn.

In paragraph 17 of the Office action, the Patent Office rejected claim 1, 3-5, 7, 11, 18-30, 32, and 37-38, alleging that the phrase "cysteine motifs of a Balbiani ring 3 protein" in claims 1, 3, 7, 18, 30, and 37 is indefinite. The Applicants respectfully traverse.

The Patent Office's first basis for rejection rests upon the two-part premise that "Since the BR3P domain is not defined in the specification, one cannot determine what a BR3P domain is." (Office action at p. 9.) Neither part of this premise is correct. The specification adequately defines the cysteine motifs of a Balbiani ring 3 protein (BR3P) at page 11, lines 16-25, citing two articles in the literature (both of record).<sup>3</sup> The citation to literature in the art is

<sup>3</sup> As discussed in paragraph 6 of the Rule 132 declaration of Dr. Alitalo dated November 26, 1997, BR3P cysteine motifs are quite distinctive in character (Cys-Xaa<sub>n</sub>-Cys-Xaa-Cys-Xaa-Cys) and occur at least four times in the

more than adequate to describe that which is already known in the art. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986) (It is axiomatic that patent applications need not contain, and preferably omit, that which is well known in the art.).<sup>4</sup>

Moreover, even if the specification lacked the description at page 11, the fact remains that the characteristic BR3P cysteine motif was within the knowledge of those skilled in the art at the time of filing, such that one skilled in the art could determine whether or not a polypeptide contained a domain characterized by one or more BR3P cysteine motifs. See M.P.E.P. § 2164.08 ("Not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted.") Thus, the patent application contains sufficient definition of cysteine motifs of a BR3P protein for the reader skilled in the art. See, e.g., *In re Moore*, 169 U.S.P.Q. 236, 238 (CCPA 1971) (Claim language must not be analyzed in a vacuum, "but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art.").

The Patent Office's second basis for rejection alleges that "it is unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter." (Office action at p. 8.) The Applicants respectfully submit that the plain language of the claims state that the claimed polypeptides lack portions *defined by the cysteines*, and that no reasonable alternative interpretation exists. For example, amended claim 1 recites, in

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carboxy terminal portion of the VEGF-C precursor polypeptide (see, e.g., SEQ ID NO: 44, Cys residues at positions 280, 291, 293, 295; residues 304, 315, 317, 319; residues 328, 339, 341, and 343; and residues 347, 358, 360, and 362). The application depicts the VEGF-C precursor amino acid sequence, and the distinctive BR3P motifs in the carboxy-terminus would be readily apparent to the reader skilled in the art.

<sup>4</sup> Notwithstanding the accepted practice of omitting that which is well known in the art, the Applicants will amend the specification to include an excerpt from the cited Dignam and Case article, if the Patent Office requests.

pertinent part, that the polypeptide "lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P)." There is no ambiguity to determining whether or not a protein's amino acid sequence includes or lacks one or more "Cys-Xaa<sub>n</sub>-Cys-Xaa-Cys-Xaa-Cys" sequences.

For all of these reasons, the rejection of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 should be withdrawn.

- C. The rejection of claims 1, 26-29, and 37 with respect to the term "high affinity" has been rendered moot.

In paragraph 18 of the Office action, the Patent Office rejected claims 1 and claims 26-29, and 37 which depend therefrom, alleging that the claims were indefinite with respect to the term "high affinity" recited in claim 1: "The term 'high affinity' is relative, and it is not clear how strongly a protein must bind to the Flt4 receptor in order for it to be considered 'high affinity.' It is suggested that the claims be amended to recite a particular range of  $K_d$ ." (Office action at p. 8.)

The Applicants respectfully submit that the term "high affinity" is not indefinite to a person of ordinary skill in the art in view of the teachings of the application and the art to which the invention pertains. Notwithstanding this fact and solely to expedite allowance, the Applicants have amended claim 1 to delete the allegedly indefinite term, rendering this basis for rejection moot. The subject matter of the claim is adequately defined by the limitations that remain after this amendment.

- D. The rejection of claims 1, 3-5, 7, 26-30, and 37 with respect to the term "including" should be withdrawn.

In paragraph 19 of the outstanding Office action, the Patent Office rejected claims 1, 3-5, 7, 26-30, and 37, alleging that the term "including" was indefinite because "it is unclear whether 'including' is equivalent to the open language 'comprising' or the closed language 'consisting of.'" (Office action at p. 9.) The Applicants respectfully traverse. The term "including" is unequivocally interpreted as open claim language, synonymous with the term

"comprising." See M.P.E.P. §2111.03. Accordingly, this rejection should be withdrawn.

- E. The rejection of claims 3, 5, 18, 24-25, and 30-31 for lack of antecedent basis has been rendered moot.

In paragraph 20 of the outstanding Office action, the Patent Office rejected claims 3, 5, 18, 24-25, and 30-31, alleging that the term "said polynucleotide" as recited in the claims lacks antecedent basis. The Applicants have amended claim 3 to provide *ipsis verbis* antecedent basis for the term "said polynucleotide," thereby rendering the rejection moot with respect to claim 3 and also claim 5 which depends from claim 3.

The Applicants have amended claims 18 and 30-31 to recite "nucleic acid" instead of "polynucleotide." This substitution of terminology renders moot the rejection of claims 18, 24-25, and 30-31. The term "nucleic acid" in the amended claims has *ipsis verbis* antecedent basis support.

For these reasons, the rejection of claims 3, 5, 18, 24-25, and 30-31 has been rendered moot, and should be withdrawn.

- F. The rejection of claim 30 has been rendered moot.

In paragraph 21 of the Office action the Patent Office alleged, "Claim 30 is indefinite with respect to the term 'VEGF-homologous portion.' It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF . . . ." (Office action at p. 9.) The Applicants respectfully submit that this phrase is clear and that "similarity" and "common evolutionary origin" are not incompatible concepts. However, solely to expedite allowance, the Applicants have deleted the allegedly indefinite term, rendering this basis for rejection moot. Accordingly, the rejection of claim 30 should be withdrawn.

**G. The rejection of claim 32 has been rendered moot.**

In paragraph 22 of the Office action, the Patent Office alleged, "Claim 32 is indefinite with respect to an amino acid sequence 'corresponding to' another amino acid sequence. It is unclear whether 'corresponding to' means that the amino acid sequence is identical or not." (Office action at p. 9.) Solely to expedite allowance, the Applicants have substituted the term "identical to" for the term "corresponding to" in claim 32, rendering this rejection moot.

**H. Conclusion.**

For all of the foregoing reasons, the Patent Office's rejections of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, should now be withdrawn.

**VI. Status update relating to priority applications.**

The 1994 priority application has now issued as U.S. Patent No. 5,776,712. The Applicants wish to apprise the Examiner that prosecution has been suspended in U.S.S.N. 08/510,133 because "A reference relevant to the examination of this application may soon become available."

**VII. Summary**

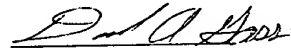
The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

Dated: July 23, 1998



David A. Gass  
Registration No. 38,153

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.

26. (Amended) A host cell according to claim 1 that expresses a naturally occurring Flt4 ligand protein encoded by said polynucleotide.

27. (Amended) A host cell according to claim 1 that expresses a human Flt4 ligand protein encoded by said polynucleotide.

28. (Amended) A host cell according to claim 1, wherein said host cell expresses said polynucleotide and produces a human protein that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, said protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. (Amended) A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

30. (Amended) A nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. (Amended) A nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.



32. (Amended) A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. (Amended) A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating phosphorylation of Flt4 receptor tyrosine kinase.

37. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

39. A method according to claim 38 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

40. A eukaryotic host cell according to claim 1 or 3 that secretes said polypeptide.

41. A nucleic acid according to claim 30 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

42. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

43. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

44. A purified and isolated nucleic acid according to claim 43 wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.



PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al. ) Title: RECEPTOR LIGAND  
Serial No: 08/585,895 ) Group Art Unit: 1646  
Filed: January 12, 1996 ) Examiner: Saoud

AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME

RECEIVED

AUG 4 1998

Assistant Commissioner for Patents  
Washington, D.C. 20231

DEPARTMENT OF COMMERCE  
SERVICE CENTER

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
3. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
4. Check in the amount of \$55.00 in payment of fee for extension of time; and
5. Check in the amount of \$159.00 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 23, 1998, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass

1. Small Entity Status

☒ Small entity status has been established and is still effective.

2. Extension of Time

☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00	X	\$55.00
Two Months		\$400.00		\$200.00
Three Months		\$950.00		\$475.00
Four Months		\$1,510.00		\$755.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid therefor of \$\_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$55.00

RECEIVED

AUG 4 1998

MAIL ROOM  
SERVICE CENTER

3. Fee for Claims

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	40	MINUS	33	7	X11 =	\$77	X22 =	\$
INDEP.	7	MINUS	6	2	X41 =	\$82	X82 =	\$
<input type="checkbox"/>	First Presentation of Multiple Dependent Claim				+ 135 =		+ 270 =	\$
TOTAL ADDITIONAL FEE						\$159	OR	\$

4. Method of Payment of Fees

☒ Attached are checks in the amount of \$55.00 and \$159.00.

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

5. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: 

David A. Gass  
Reg. No: 38,153

July 23, 1998



**PATENT**  
**28967/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s): Alitalo et al. ) Title: RECEPTOR LIGAND  
Serial No: 08/585,895 ) Group Art Unit: 1646  
Filed: January 12, 1996 ) Examiner: Saoud  
)  
)

**AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME**

RECEIVED

AUG 4 1998

RECEIVED  
SERVICE CENTER

**Assistant Commissioner for Patents**  
**Washington, D.C. 20231**

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David A. Gass

1. Small Entity Status

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EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00	X	\$55.00
Two Months		\$400.00		\$200.00
Three Months		\$950.00		\$475.00
Four Months		\$1,510.00		\$755.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid therefor of \$\_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$55.00



3. Fee for Claims

The fee for additional claims ([37 CFR 1.16(b)-(d)] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	40	MINUS	33	7	X11 =	\$77	X22 =	\$
INDEP.	7	MINUS	5	2	X41 =	\$82	X82 =	\$
<input type="checkbox"/>	First Presentation of Multiple Dependent Claim				+135 =		+270 =	\$
TOTAL ADDITIONAL FEE						\$159	OR	\$

4. Method of Payment of Fees

- ☒ Attached are checks in the amount of \$55.00 and \$159.00.
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

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Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By:

  
David A. Gass  
Reg. No: 38,153

July 23, 1998

Jul 23, 1998 5:15PM MARSHALL, OTOOLE

No. 8729 P. 1/4  
From: 0819

**MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN**

ATTORNEYS AT LAW  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO, ILLINOIS 60606-6402  
(312) 474-6300  
FAX: (312) 474-0448

July 23, 1998

**FACSIMILE TRANSMITTAL SHEET**

**TO:** Examiner Saoud - Group Art Unit: 1646  
c/o U.S. Patent and Trademark Office  
(703) 308-0294  
U.S. Serial No. 08/585,895

**CLIENT NO:** 28967  
**MATTER NO:** 33072  
**COUNTRY CODE:** US

**FROM:** David A. Gass  
Marshall, O'Toole

**PAGES (INCLUDING THIS PAGE):** 4

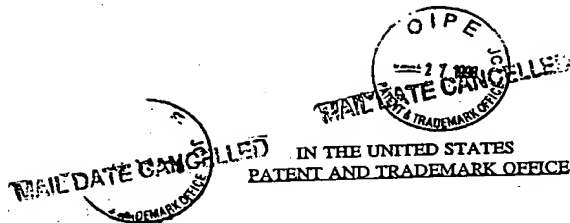
**PLEASE CONFIRM RECEIPT:** No.

**MESSAGE:** The attached declaration, which is a duplicate of a declaration filed November 26, 1997, was referenced in amendment papers filed today by first class mail in the above matter, but may have been inadvertently omitted.

*Please contact Lisa Richard at (312) 474-6819 if you do not receive all of the pages in good condition.*

\*\*\*\*\*

*The material of this transmission contains confidential information intended only for the addressee. If you are not the addressee, any disclosure or use of this information by you is strictly prohibited. If you have received this facsimile in error, please notify us by telephone immediately.*



PATENT  
28967/33072



In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Art Unit: 1646

Examiner: Saoud

I hereby certify that this paper is  
deposited with the United States Postal  
Service as first class mail, postage  
prepaid, in an envelope addressed to:  
Assistant Commissioner for Patents  
Washington, D.C. 20231, on this date:

Dated: July 23, 1998

  
David A. Cass

#### DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

I, Kari Alitalo, do hereby declare and state as follows:

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated March 24, 1998, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

2. I understand that sequences identified as SEQ ID NOs: 44 and 45 were added to the patent application by an amendment dated November 26, 1997, and entered by the Patent Office on December 1, 1997. Copies of those two sequences are appended hereto. I understand that, at the time of the amendment, SEQ ID NOs: 44 and 45 were identified as a nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the American Type Culture Collection (ATCC) as plasmid pFLT4-L and that is cross-referenced in the patent application at pages 28-29. I understand that the Patent Office has objected to the amendment to introduce these two sequences into the patent application on the

basis that such an amendment "introduces new matter into the disclosure." The Patent Office's basis for this allegation was as follows:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "the approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44."

(Office action dated March 24, 1998, at paragraph 10.)

3. I confirm that our laboratory sequenced the insert of the same plasmid that was designated pFLT4-L and that was deposited with the ATCC as ATCC Deposit No. 97231 and that is referred to at pages 28-29 of the patent application. The nucleotide sequence of the insert of this plasmid (ATCC Deposit No. 97231) includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44 as appended hereto and added to the patent application in the amendment dated November 26, 1997. The 419 residue amino acid sequence set forth in SEQ ID NO: 45 (as appended hereto and added to the patent application) is deduced from the sequence set forth in SEQ ID NO: 44.

4. The insert of plasmid pFLT4-L (ATCC Deposit No. 97231) contains additional (non-coding) sequence adjacent to the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The apparent size discrepancy between the approximately 2.1 kb size of the insert (as estimated by agarose gel electrophoresis analysis) and the 1997 nucleotides of sequence as set forth in SEQ ID NO: 44 is explained by the existence of this additional non-coding sequence in the plasmid insert.

#### Certification

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and

the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

July 17, 1998  
Date

Kari Alitalo  
Kari Alitalo

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCCCCCCCCG CTCCTCAAAA AGCTACACCG ACGCGGACCG CGGCGGGGTC CTCCTTOGCC	60
CTCGCITCAC CTCGCGGCT CCGAATCGGG GGAGCTCGGA TGTCCGGTIT CCTGTGAGGC	120
TTTTACCTGA CACCCGCCGC CTITCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG	180
GGAACGCGGA GCCCCGGACC CGCTCCCGCC GCTTCGGGCT CGCCAGGGG GGGTCGCCGG	240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC	300
CCACCCCTGC CCCC GCCAGG GGACCGGTC CCCACCCCG GTCTTTCAC C ATG CAC	357
Met His	
1	
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG	405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu	
5 10 15	
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC	453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser	
20 25 30	
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT	501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala	
35 40 45 50	
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA	549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val	
55 60 65	
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG	597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys	
70 75 80	
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC	645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn	
85 90 95	

CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	693
Leu Asn Ser Arg Thr Glu Thr Ile Lys Phe Ala Ala Ala His Tyr	
100 105 110	
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	
115 120 125 130	
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	
135 140 145	
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	837
Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys	
150 155 160	
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG	885
Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr	
165 170 175	
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA	933
Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln	
180 185 190	
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA	981
Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg	
195 200 205 210	
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA	1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg	
215 220 225	
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC	1077
Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr	
230 235 240	
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT	1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala	
245 250 255	
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT	1173
Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp	
260 265 270	
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC	1221
Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr	
275 280 285 290	
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC	1269
Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro	
295 300 305	
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA	1317
His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys	
310 315 320	

CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA 1365  
 Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr  
 325 330 335

TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT 1413  
 Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn  
 340 345 350

CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG 1461  
 Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu  
 355 360 365 370

TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG 1509  
 Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg  
 375 380 385

CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT 1557  
 Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser  
 390 395 400

GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG 1605  
 Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met  
 405 410 415

AGC TAAGATTGTA CTGTTTCCCA GTTCATCGAT TTCTATTAT GGAAACTGT 1658  
 Ser

GTGCCACAG TAGAACTGTC TGGAACAGA GAGACCTTG TGGTCCATG CTAACAAAGA 1718

CAAAAGTCTG TCCTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC 1778

TGCAAAAGGC CTCCTGTAAA GACTGGTTTT CTGCCAATGA CCAACAGCC AAGATTTTCC 1838

TCCTGTGATT TCCTTAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT 1898

GCATTCATT TTATAGCAAC AACAAATGGT AAAACTCACT GTGATCAATA TTTTATATC 1958

ATGCAAAATA TGTTAAAT AAAATGAAAA TTGTATTAT 1997

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 419 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala  
 1 5 10 15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe  
 20 25 30



Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala  
 35 40 45  
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser  
 50 55 60  
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met  
 65 70 75 80  
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln  
 85 90 95  
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala  
 100 105 110  
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys  
 115 120 125  
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe  
 130 135 140  
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr  
 145 150 155 160  
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr  
 165 170 175  
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu  
 180 185 190  
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser  
 195 200 205  
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile  
 210 215 220  
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn  
 225 230 235 240  
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys  
 245 250 255  
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser  
 260 265 270  
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu  
 275 280 285  
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys  
 290 295 300  
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys  
 305 310 315 320  
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu  
 325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro  
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys  
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr  
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser  
385 390 395 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro  
405 410 415

Gln Met Ser



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/085,895	01/12/98	ALITALIA	K 18113/33072

MARSHALL, GYDOUEL KERSTEIN,  
MURRAY & BORJIN  
6000 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO, IL 60606-6482

EXAMINER
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ART. UNIT.	PAPER NUMBER
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27  
10/08/98

DATE MAILED:

Please find below a communication from the EXAMINER in charge of this application.

Commissioner of Patents

Applicant's response filed 27 July 1998 has been received. However, a reference relevant to the examination of this application may soon become available. *Ex parte* prosecution is SUSPENDED INDEFINITELY from the date of this letter. Applicant should feel free to make an inquiry as to the status of the application if needed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Friday from 8AM to 3PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lila Feisee, can be reached on (703) 308-2731. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4227. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Christine Saoud, Ph.D.  
October 6, 1998

JOHN ULM  
PRIMARY EXAMINER  
GROUP 1800





PATENT  
Attorney Docket No. 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

JUL 24 1999

In the Application of: Kari Alitalo

and Vladimir Joukov

Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: Saoud, C.

) I hereby certify that this paper and  
) the documents referred to as  
) enclosed herewith are being  
) deposited with the United States  
) Postal Service as First Class Mail,  
) postage prepaid, in an envelope  
) addressed to: Assistant  
) Commissioner for Patents,  
) Washington, DC 20231, on this  
) date:

) July 26, 1999

) Jill E. Uhl  
) Jill E. Uhl

) Reg. No.: 43,213

) Attorney for Applicants

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Submitted herewith are a Form PTO-1449 listing several documents, together with a copy of each listed document. The Applicants respectfully request that these documents be made of record and considered in the above-identified application.

Documents A3-A6 are U.S. priority documents of published PCT applications that are now publically available from WIPO.

Documents B9-B11, C117, C119, and C154-C157 were identified by the European Patent Office in an International Search Report for a related PCT application. A

copy of the search report is also attached hereto.

Documents C120-C153 pertain to sequences, such as EST's, that have been posted in the Genbank Database, where the sequences should be available in computer readable form.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

Please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

July 26, 1999

By:

Jill E. Uhl  
Jill E. Uhl  
Registration No.: 43,213  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



**PATENT**

Attorney Docket No. 28967/33072

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In the Application of: Kari Alitalo	)	I hereby certify that this paper and
	)	the documents referred to as
and Vladimir Joukov	)	enclosed herewith are being
	)	deposited with the United States
Serial No.: 08/585,895	)	Postal Service as First Class Mail,
	)	postage prepaid, in an envelope
Filed: January 12, 1996	)	addressed to: Assistant
	)	Commissioner for Patents,
For: RECEPTOR LIGAND	)	Washington, DC 20231, on this
	)	date:
Group Art Unit: 1646	)	
	)	July 26, 1999
Examiner: Saoud, C.	)	
	)	<i>Jill E. Uhl</i>
	)	Jill E. Uhl
	)	Reg. No.: 43,213
	)	Attorney for Applicants

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July 26, 1999

By: Jill E. Uhl

Jill E. Uhl  
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233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



# FILE COPY

#28

SHEET 1 of 5

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. et al.	
		Filing Date January 12, 1996	Group 1646

<del>U.S. PATENT DOCUMENTS</del>							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
OK	A3	08/207,550	none	Jing-Shan Hu and Liang Cao			03/08/94
OK	A4	08/465,968	none	Crain Rosen, Jing- Shan Hu and Liang Cao			06/06/95
OK	A5	60/003,491	none	James Lee and William Wood			09/08/95
OK	A6	08/554,374	none	Lyman, S.			11/08/95

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
OK	B8	0 506 477 A1	03/27/92	EP				
OK	B9	97/05250 A	02/13/97	WO				
OK	B10	97/09427 A	03/13/97	WO				
OK	B11	97/17442 A	05/15/97	WO				

EXAMINER <i>C. Saoud</i>	DATE CONSIDERED <i>3/28/00</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

SHEET 2 of 2

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		Filing Date January 12, 1996	Group 1646

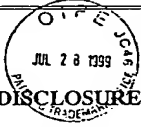
OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
u	C117	Achen, M.G. et al., "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," <i>Proceedings of the National Academy of Science, USA</i> , 95:548-553 (January, 1998).
	C118	Adams, M.D. et al., "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," <i>Nature</i> , 377(6547 Supplement):3-174 (September, 1995).
	C119	Cohen, T. et al., "VEGF <sub>121</sub> , A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells," <i>Journal of Biological Chemistry</i> , 270(19):11322-11326 (May 12, 1995).
	C120	Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 3'," Hillier, L. et al., Dated 14-May-1997
	C121	Genbank AA425486, "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 5' similar to SW:VEGF MOUSE Q00731 VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR," Deposited by Hillier, L. et al. Dated 16-Oct-1997
	C122	Genbank N31713, "yy15b12.s1 Homo sapiens cDNA clone 271295 3'," Deposited by Hillier, L. et al. Dated 10-Jan-1996
	C123	Genbank N31720, "yy15d12.s1 Homo sapiens cDNA clone 271319 3'," Deposited by Hillier, L. et al. Dated 10-Jan-1996
	C124	Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 75366 5'," Deposited by Hillier, L. et al. Dated 17-May-1997
	C125	Genbank N50972, "yy94b08.s1 Homo sapiens cDNA clone 281175 3'," Deposited by Hillier, L. et al. Dated 14-Feb-1996
u	C126	Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 738893 3'," Deposited by Hillier, L. et al. Dated 16-Oct-1997

EXAMINER C. Saund	DATE CONSIDERED 3/28/00
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<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date January 12, 1996	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
✓	C127	Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 309535 3'," Deposited by Hillier, L <i>et al.</i> Dated 20-Aug-1996
	C128	Genbank H05177, "y185b08.r1 Homo sapiens cDNA clone 44993 5'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995
	C129	Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 3'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997
	C130	Genbank H05134, " y185b08.s1 Homo sapiens cDNA clone 44993 3'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995
	C131	Genbank, AA298182 "EST113866 Bone VII Homo sapiens cDNA 5' end," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997
	C132	Genbank AA298283, "EST113896 Bone VII Homo sapiens cDNA 5' end similar to similar to vascular endothelial growth factor," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997
	C133	Genbank T81481, "yd29f07.s1 Homo sapiens cDNA clone 109669 3'," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
	C134	Genbank AA425303, "zw46b06.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 3', mRNA sequence," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997
	C135	Genbank Z40230, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995
	C136	Genbank Z44272, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995
	C137	Genbank AA478766, " zv18h12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 5'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997
✗	C138	Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 3'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996

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 <b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date January 12, 1996	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
CA	C139	Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996
	C140	Genbank T81690, "yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
	C141	Genbank T84377, "yd37h08.r1 Homo sapiens cDNA clone 110463 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 16-Mar-1995
	C142	Genbank N42368, "yy15b11.r1 Homo sapiens cDNA clone 271293 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
	C143	Genbank N42374, "yy15d11.r1 Homo sapiens cDNA clone 271317 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
	C144	Genbank H81868, "yv83d09.s1 Homo sapiens cDNA clone 249329 3'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
	C145	Genbank H81867, "yv83d09.r1 Homo sapiens cDNA clone 249329 5'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
	C146	Genbank AA149461, "z127h03.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 5' similar to SW:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3 PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 14-May-1997
	C147	Genbank R77495, "yi79e04.s1 Homo sapiens cDNA clone 145470 3'," Deposited by Hillier, L. <i>et al.</i> Dated 07-Jun-1995
	C148	Genbank H07899, "y186g06.s1 Homo sapiens cDNA clone 45138 3'," Deposited by Hillier, L. <i>et al.</i> Dated 23-Jun-1995
	C149	Genbank T89295, "yd37h08.s1 Homo sapiens cDNA clone 110463 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Mar-1995
UN	C150	Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence," Deposited by Okubo, K. Dated 01-Oct-1996

EXAMINER C. Saoud	DATE CONSIDERED 3/28/96
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Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date January 12, 1996	Group 1646

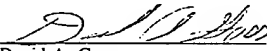
OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
✓	C151	Genbank N82975, "TgESTzy53h10.r1 TgRH Tachyzoite cDNA Toxoplasma gondii cDNA clone tgzy53h10.r1 5'," Deposited by Hehl, A. <i>et al.</i> Dated 10-Sep-1997
	C152	Genbank AA285997, "vb88h06.r1 Soares mouse 3NbMS Mus musculus cDNA clone 764123 5'," Deposited by Marra, M. <i>et al.</i> Dated 09-Apr-1997
	C153	Genbank AA549856, "0929m3 gmbPfHB3.1, G. Roman Reddy Plasmodium falciparum genomic clone 0929m," Deposited by Dame, J.B. <i>et al.</i> Dated 11-Aug-1997
	C154	Jeltsch, M. <i>et al.</i> , "Hyperplasia of Lymphatic Vessels in VEGF-C Transgenic Mice," <i>Science</i> , 276:1423-1425 (May, 1997).
	C155	Joukov, V. <i>et al.</i> , "Proteolytic Processing Regulates Receptor Specificity and Activity of VEGF-C," <i>EMBO Journal</i> , 16(13):3898-3911 (June, 1997).
	C156	Joukov, V. <i>et al.</i> , "A Recombinant Mutant Vascular Endothelial Growth Factor-C that has Lost Vascular Endothelial Growth Factor Receptor-2 Binding, Activation, and Vascular Permeability Activities," <i>Journal of Biological Chemistry</i> , 273(12):6599-6602 (March 20, 1998).
✓	C157	Lee, J. <i>et al.</i> , "Vascular Endothelial Growth Factor Related Protein (vrp): A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," EMBL Sequence Data Library, XP002066361, accession no. U4142. Dated 10-Jan-1996

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RECEIVED  
NOV 16 1996  
PATENT  
Attorney Docket No. 28967/33072

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	)	Washington, DC 20231, on this date:
For: RECEPTOR LIGAND	)	
	)	October 26, 1999
Group Art Unit: 1646	)	
	)	
Examiner: Saoud, C.	)	
	)	David A. Gass
	)	Reg. No.: 38,153
	)	Attorney for Applicants

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
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Assistant Commissioner for Patents  
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Sir:

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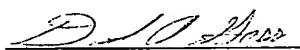
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(312) 474-6300

October 26, 1999

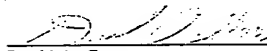
By:

  
David A. Gass  
Reg. No.: 38,153



**PATENT**  
Attorney Docket No. 28967/33072

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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	)	
Examiner: Saoud, C.	)	
	)	David A. Gass
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	)	Attorney for Applicants

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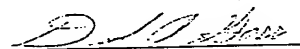
Pursuant to 37 C.F.R. §1.97(e)(2), the listed documents were not known to the applicants or to any individual designated in §1.56 (c) as issued U.S. patents more than three months prior to the filing of this Supplemental Information Disclosure Statement, because U.S. Patent No. 5,932,540 (document A7) issued on August 3, 1999 and U.S. Patent No. 5,935,820 (document A8) issued on August 10, 1999. Consequently, this Supplemental Information Disclosure Statement should be considered by the Patent Office without payment of a fee. However, please charge any necessary fees due in connection with this Supplemental Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

October 26, 1999

By:

  
David A. Gass  
Reg. No.: 38,153

# FILE COPY

#2  
SHEET 1 of 1

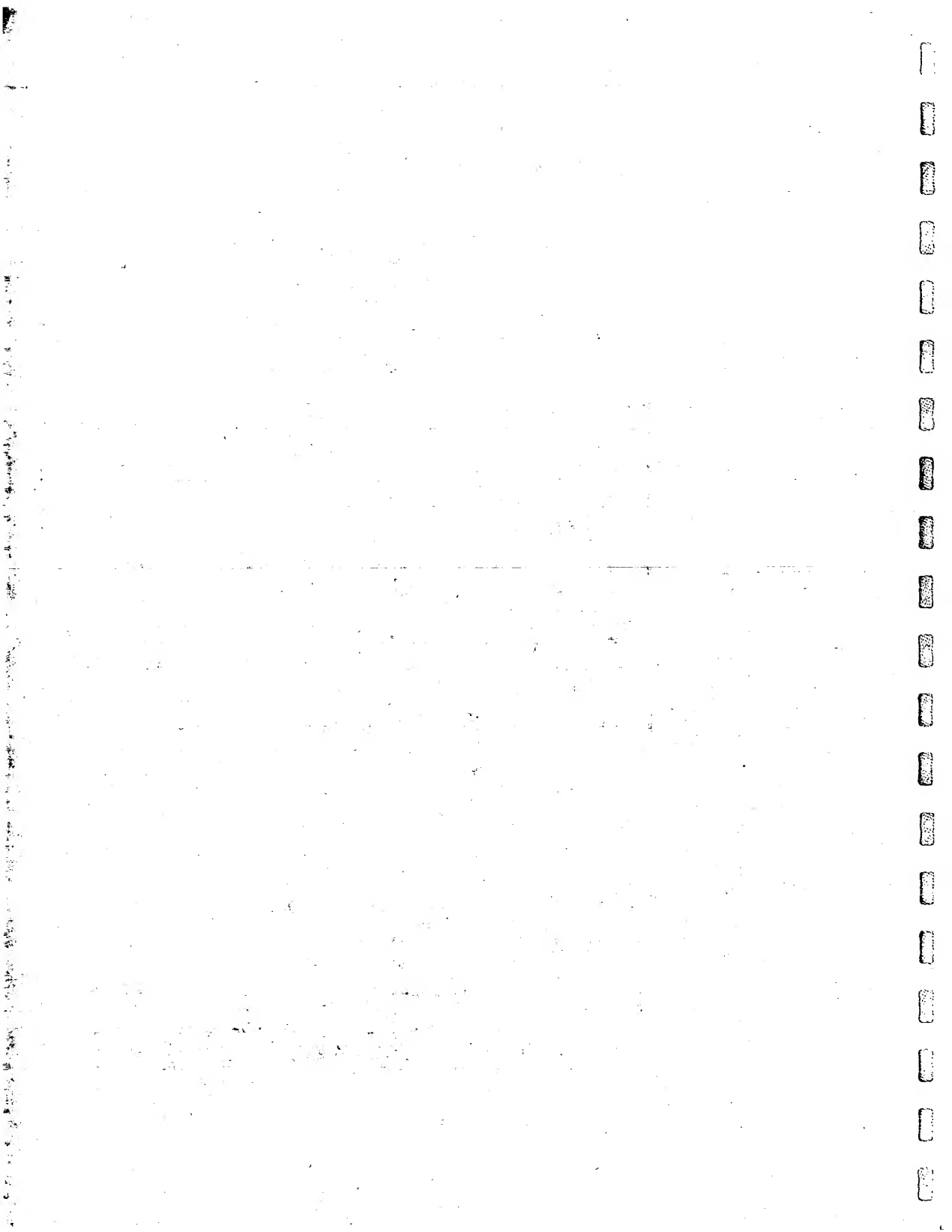
Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. et al.	
		Filing Date January 12, 1996	Group 1646

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
CK	A7	5,932,540	08/03/99	Jing-Shan Hu et al.	514	2	
CK	A8	5,935,820	08/10/99	Jing-Shan Hu et al.	435	69.4	

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		

EXAMINER C. Saoud	DATE CONSIDERED 3/28/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	





UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/585,895	01/12/96	ALITALO	K 28113/33072

HM22/0404  
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

EXAMINER

SAOUD, C

ART UNIT	PAPER NUMBER
1646	30

DATE MAILED: 04/04/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

# Office Action Summary

Application No.  
08/585,895

Applicant(s)

Alitalo et al.

Examiner

Christine Seoud

Group Art Unit  
1646

☐ Responsive to communication(s) filed on \_\_\_\_\_

☐ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

## Disposition of Claims

☒ Claim(s) 1, 3-5, 7, 11, and 18-44 is/are pending in the application.

Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.

☐ Claim(s) \_\_\_\_\_ is/are allowed.

☒ Claim(s) 1, 3-5, 7, 11, and 18-44 is/are rejected.

☐ Claim(s) \_\_\_\_\_ is/are objected to.

☐ Claims \_\_\_\_\_ are subject to restriction or election requirement.

## Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

## Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been received.

☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

## Attachment(s)

☐ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 28 and 29

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

Application/Control Number: 08/585,895

Page 2

Art Unit: 1646

#### DETAILED ACTION

##### *Response to Amendment*

1. Claims 1, 3-5, 7, 18, 19, 26-33, and 36-37 have been amended and claims 39-44 have been added as requested in the amendment of paper #26, filed 27 July 1998. Claims 1, 3-5, 7, 11, and 18-44 are pending in the instant application.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Any objection or rejection of record which is not expressly repeated in this action has been overcome by Applicant's response and withdrawn.
4. Applicant's arguments filed 27 July 1998 have been fully considered, however, in light of the new grounds of rejection below, the arguments are not found to be relevant and therefore, have not been addressed.

##### *Claim Rejections - 35 USC § 112*

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

Art Unit: 1646

make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1, 37, and 42 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1 and 42 and dependent claim 37 are directed to subject matter of a polynucleotide that hybridizes to a DNA under specific conditions which are recited in the claims, wherein the polynucleotide encodes a protein which has particular structural and functional features. In making a determination of whether the application complies with the written description requirement of 35 U.S.C. 112, first paragraph, it is necessary to understand what Applicant has possession of and what Applicant is claiming. From the specification, it is clear that Applicant has possession of a nucleic acid molecule which encodes a protein which has the amino acid sequence of SEQ ID NO:33. This nucleic acid molecule has a nucleic acid sequence of SEQ ID NO:32 and is contained within plasmid pFLT4-L (ATCC deposit #97231). The subject matter which is claimed is described above. First, a determination of the level of predictability in the art must be made in that whether the level of skill in the art leads to a predictability of structure; and/or whether teachings in the application or prior art lead to a predictability of structure. The claims are directed to host cells which are transfected with a polynucleotide which encodes a polypeptide, wherein the polynucleotide hybridizes to a DNA of SEQ ID NO:32 under a specific set of hybridization conditions. First, the claims are not limited to polynucleotide molecules



Art Unit: 1646

encoding a protein with a specific amino acid sequence. The claims only require the nucleic acid molecule to encode a polypeptide which belongs to the VEGF/PDGF family (implied by the recitation of the 8 cysteine domain) and which is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The specification only describes a single polypeptide from a human and fails to teach or describe any other polypeptide which has the structural and functional characteristics recited in the claims. The breadth of the claims is such that the claims encompass polynucleotides from other species and polynucleotides which encode variant polypeptides so long as receptor binding activity is maintained. There is a lack of guidance or teaching regarding structure and function because there is only a single example provided in the specification and because there is no guidance found in the prior art. The claims include polynucleotides which share some sequence similarity to the disclosed polynucleotide which encodes the polypeptide of SEQ ID NO:33, however, this sequence similarity is not sufficient to provide the function of encoding a polypeptide which binds to the Flt4 receptor tyrosine kinase.

Next in making a determination of whether the application complies with the written description requirement of 35 U.S.C. 112, first paragraph, each claimed species and genus must be evaluated to determine whether there is sufficient written description to inform a skilled artisan that applicant was in possession of the claimed invention at the time the application was filed. With this regard, the instant application fails to provide a written description of the species or the genus which are encompassed by the instant claims except for the nucleic acid of SEQ ID NO:32. The specification does not provide a complete structure of those polynucleotides which encode a

Application/Control Number: 08/585,895

Page 5

Art Unit: 1646

polypeptide as described in the claims and hybridize to the recited sequence under the recited stringency conditions of the claims. The claims also fail to recite other relevant identifying characteristics (physical and/or chemical and/or functional characteristics coupled with a known or disclosed correlation between function and structure) sufficient to describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention. The specification fails to provide a representative number of species for the claimed genus (those polynucleotides which hybridize to SEQ ID NO:32 under the recited stringency conditions) because the claims are directed to those polynucleotides which encode a polypeptide having a conserved cysteine domain and which binds to the human Flt4 receptor tyrosine kinase, which encompasses different species and variants and the specification teaches one embodiment. Therefore, the claims are directed subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

*Claim Rejections - 35 USC § 102*

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(c) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who

Art Unit: 1646

has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

8. Claims 1, 3-5, 7, 11, and 18-44 are rejected under 35 U.S.C. 102(e) as being anticipated by Hu et al. (U.S. Pat. No. 5,935,820).

Hu et al. disclose a polynucleotide, SEQ ID NO:1, which encodes a polypeptide, SEQ ID NO:2, which includes a domain defined by 8 cysteine residues of the VEGF family, and which is capable of binding to human Flt4 receptor tyrosine kinase. The instant claims indicate that the polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein, however, this limitation only further defines the processed protein and places no material limitations on the polynucleotide. Claim 11 further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide. Claim 18 is directed to a polynucleotide which lacks a portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a "polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" anticipates this claim.

*Allowable Subject Matter*

9. It is noted that some of the claims appear to be directed to polynucleotides encoding a polypeptide comprising amino acids 1-120 of SEQ ID NO:33. The prior art does not disclose or

Application/Control Number: 08/585,895

Page 7

Art Unit: 1646

teach a polypeptide consisting of amino acids 1-120 of SEQ ID NO:33. Specific claims to the embodiment of polynucleotide encoding a polypeptide consisting of amino acids 1-120 of SEQ ID NO:33 appear to be free of the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Friday from 8AM to 3PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz, can be reached on (703) 308-4623. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4227. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

April 3, 2000

**CHRISTINE SAOUD  
PATENT EXAMINER**

*Christine Saoud*

PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al. )  
Serial No: 08/585,895 )  
Filed: January 12, 1996 )  
Title: Receptor Ligand )  
Group Art Unit: 1646 )  
Examiner: Christine Saoud )

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The undersigned attorney of record in the above-identified application  
hereby appoints as associate attorney(s):

Frank S. DiGiglio (Reg. No. 31,346)  
Scully, Scott, Murphy & Presser  
400 Garden City Plaza  
Garden City, New York 11530  
(516) 742-4343

to prosecute this application, to make alterations or amendments therein, and to  
transact any and all business in the Patent and Trademark Office connected  
therewith.

MARSHALL, OTOOLE, GERSTEIN,  
MURRAY & BORUN



David A. Gass  
Registration No. 38,153

June 22, 2000





UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/585,895	01/12/95	AI ITALIA	28113/34072

HM22/0629

FRANK S. DIGIELLO  
SCULLY SCOTT MURPHY & PRESSER  
400 GARDEN CITY PLAZA  
GARDEN CITY NY 11530

EXAMINER

SADUD, C

ART UNIT

1647

PAPER NUMBER

32

DATE MAILED: 06/29/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILED DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/585,895			

EXAMINER
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ART UNIT	PAPER NUMBER
----------	--------------

31

DATE MAILED:

#### INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Christine Saoud (3) DAVID GASS  
(2) GARY KUNZ (4) WILLIAM MIERHEL  
Date of Interview June 22, 2000 (5) FRANK J. DiGuglio

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description:

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: 1, 3, 33, 18

Identification of prior art discussed: Hu et al. - of record in last office action.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed clms w/ hybridization language as it relates to written description and enablement for prot. which binds Fil 4. Discussed host cell clms and product of mature (truncated) VEGF-C. Host cells which produce mature VEGF-C distinguish over the prior art of record.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM PTOL-413 (REV. 1-99)

Christine Saoud





SAU 1646 RECEIVED

AUG 15 2000

TECH CENTER 1600/2500

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): ) Title: RECEPTOR LIGAND  
Alitalo et al. )  
Serial No: 08/585,895 ) Group Art Unit: 1646  
Filed: January 12, 1996 ) Examiner: Christine Saoud

AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo (unsigned); and
3. Check in the amount of \$91.00 in payment of fee for extension of time (\$55.00) and fee for extra claims (\$36.00).

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 4, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass

08/11/2000 JADD01 00000011 08585895

01 FC:215

\$5.00 DP

RECEIVED  
 JUN 11 1991  
 TRADEMARK

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.  
☐ Has not been established.

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For	RATE	FEE	RATE	FEE
TOTAL	44	MINUS 40 = 4	X 9 =	\$36.00	X 18 =	\$
INDEP.	7	MINUS 7 = 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 130 =	\$	+ 260 =	\$
Filing Fee:				\$36.00	OR	\$

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00	X	\$55.00
Two Months		\$380.00		\$190.00
Three Months		\$870.00		\$435.00
Four Months		\$1,360.00		\$680.00
Five Months		\$1,850.00		\$925.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

- ☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid thereof of \$ \_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

RECEIVED

AUG 15 2000

TECH CENTER 1600/2

Extension Fee Due With This Request: \$55.00

3. Method of Payment of Fees

- ☒ Attached is a check in the amount of \$91.00
- ☐ Charge Deposit Account No. 13-2855  
in the amount of: \$ \_\_\_\_\_  
A copy of this Petition is enclosed.

4. Deposit Account and Refund Authorization

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: 

David A. Gass  
Reg. No: 38,153

August 4, 2000



RECEIVED  
AUG 10 2000  
TECH CENTER  
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	)	Title: RECEPTOR LIGAND
Alitalo et al.	)	
Serial No: 08/585,895	)	Group Art Unit: 1646
Filed: January 12, 1996	)	Examiner: Christine Saoud

AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents  
Washington, D.C. 20231

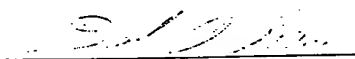
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2. Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo (unsigned); and
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David A. Gass

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 Has not been established.

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For	RATE	FEE	RATE	FEE
TOTAL	44	MINUS 40 = 4	X 9 =	\$36.00	X 18 =	\$
INDEP.	7	MINUS 7 = 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 130 =	\$	+ 260 =	\$
Filing Fee:				\$36.00	OR	\$

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EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
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Four Months		\$1,360.00		\$680.00
Five Months		\$1,850.00		\$925.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: **\$55.00**

- ☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid therefor of \$ \_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

Extension Fee Due With This Request: \$55.00

3. Method of Payment of Fees

- ☒ Attached is a check in the amount of \$91.00
- ☐ Charge Deposit Account No. 13-2855  
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MARSHALL, O'TOOLE, GERSTEIN,  
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6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: \_\_\_\_\_

David A. Gass  
Reg. No: 38,153

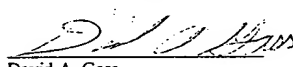
August 4, 2000



E/35  
7/27  
8/15/00

PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.	)	I hereby certify that this paper is being
Serial No: 08/585,895	)	deposited with the United States Postal
Filed: January 12, 1996	)	Service with sufficient postage as first class
Title: RECEPTOR LIGAND	)	mail, postage prepaid, in an envelope
	)	addressed to: Assistant Commissioner for
	)	Patents, Washington, D.C., 20231 on this
	)	date:
	)	
	)	Date: August 4, 2000
Group Art Unit: 1646	)	
Examiner: Christine Saoud	)	
	)	David A. Gass
	)	Registration No. 38,153
	)	Attorney for Applicants
	)	

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

In an Office action mailed April 4, 2000, the Patent Office rejected claims 1, 3-5, 7, 11, and 18-44 variously under 35 USC §§ 102(e) and 112, first paragraph. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This amendment is timely filed with a petition and fee for one month extension of time.

08/11/2000 JADD01 00000011 08585895

02 FC:203

36.00 DP

## AMENDMENTS

### In the claims:

Please cancel all pending claims and add new claims 45-79 as shown below:

~~40.~~ <sup>4</sup> A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds to human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

~~46.~~ <sup>2</sup> A purified and isolated nucleic acid according to claim ~~45~~ <sup>1</sup> wherein said polypeptide stimulates tyrosine phosphorylation of human Flt4.

~~47.~~ <sup>3</sup> A purified and isolated nucleic acid according to claim ~~46~~ <sup>2</sup> wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

~~48.~~ <sup>4</sup> A purified and isolated nucleic acid according to claim ~~47~~ <sup>3</sup> wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

~~49.~~ <sup>5</sup> A purified and isolated nucleic acid according to claim ~~48~~ <sup>4</sup> wherein said polypeptide comprises approximately 120 amino acids.

~~50.~~ <sup>6</sup> A purified and isolated nucleic acid according to claim ~~49~~ <sup>4</sup> wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

~~51.~~ <sup>7</sup> A purified and isolated nucleic acid according to claim ~~50~~ <sup>2</sup> wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.



8  
52. A purified and isolated nucleic acid according to claim 48 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

9  
53. A nucleic acid according to claim 48 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

10  
54. A nucleic acid according to claim 48 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

11  
55. A nucleic acid according to claim 48 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

12  
56. A vector comprising a nucleic acid according to claim 48, wherein said vector lacks a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

13  
57. A host cell transformed or transfected with a vector according to claim 12.

14  
58. A method for producing a polypeptide that binds to the extracellular domain of human Flt4, comprising the steps of:  
growing a host cell according to claim 57 under conditions which permit expression by said host cell of a polypeptide that is encoded by said nucleic acid and that binds to the extracellular domain of human Flt4; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

<sup>15</sup>  
~~58.~~ A method according to claim <sup>14</sup>~~58~~ wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

<sup>16</sup>  
~~60.~~ A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

<sup>17</sup>  
~~61.~~ A purified and isolated nucleic acid according to claim <sup>16</sup>~~60~~ wherein said encoded polypeptide stimulates tyrosine phosphorylation of human Flt4.

<sup>18</sup>  
~~62.~~ A purified and isolated nucleic acid according to claim <sup>16</sup>~~60~~ wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.

<sup>19</sup>  
~~63.~~ An expression construct comprising the nucleic acid according to claim <sup>18</sup>~~62~~ operatively linked to an expression control sequence, said expression construct lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

<sup>19</sup>  
~~64.~~ A host cell transformed or transfected with the expression construct of claim <sup>19</sup>~~63~~.

<sup>21</sup>  
~~65.~~ A method for producing a polypeptide that binds to the extracellular domain of human Flt4 and stimulates tyrosine phosphorylation of Flt4, comprising the steps of:

growing a host cell according to claim 64 under conditions which permit expression in said host cell of a polypeptide encoded by said nucleic acid and isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide binds to the extracellular domain of human Flt4 and stimulates phosphorylation of Flt4.

22/6. A host cell transformed or transfected with a polynucleotide, wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

21 wherein said host cell expresses a polypeptide encoded by said polynucleotide, wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions and includes a domain encoded by the human nucleotide sequence that is defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide binds to the extracellular domain of human Flt4 receptor tyrosine kinase.

23 22/6. A host cell according to claim 64 that expresses a naturally occurring human Flt4 ligand polypeptide encoded by said polynucleotide.

24  
66. A host cell according to claim 66 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

25  
67. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC),

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that precedes position 1 and lacks any portion of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS PAGE under reducing conditions and binds to Flt4EC.

26  
76. A host cell according to claim 25 wherein said nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

27  
77. A host cell according to claim 25 wherein said polynucleotide is a vector comprising an expression control sequence operatively linked to the nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33.

28  
78. A eukaryotic host cell according to claim 22 or 25 that secretes said polypeptide.

29  
79. A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert,

wherein said secreted polypeptide has a molecular weight of about 23kD as assessed by SDS-PAGE under reducing conditions and binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

30. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that binds to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

21 (b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said expressed and secreted polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

31. A method for producing a polypeptide that binds the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 28, 29, 30, or 31 under conditions which permit expression by said host cell of said polypeptide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

<sup>32</sup><sub>32</sub> A method for producing a polypeptide that binds to the extracellular domain (EC) of human Flt4 receptor tyrosine kinase (Flt4), comprising steps of:  
growing a host cell comprising a polynucleotide that comprises a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:33, under conditions in which the host cell expresses and secretes a polypeptide encoded by the polynucleotide; and  
isolating a polypeptide that binds Flt4 EC from the growth medium of the host cell, said polypeptide having a molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and having an amino acid sequence comprising a portion of SEQ ID NO:33 effective to bind Flt4 EC.

<sup>33</sup><sub>33</sub> A method according to claim <sup>32</sup><sub>32</sub> wherein said polynucleotide comprises an expression vector that comprises a nucleotide sequence that encodes the amino acid set forth in SEQ ID NO:33.

<sup>34</sup><sub>34</sub> A method according to claim <sup>32</sup><sub>32</sub> wherein said host cell comprises a PC-3 prostatic adenocarcinoma cell (ATCC CRL1435).

<sup>35</sup><sub>35</sub> A method according to claim <sup>32</sup><sub>32</sub> wherein said polynucleotide comprises the insert of plasma pFLT4-L, deposited as ATCC Accession No. 97231.-

#### REMARKS

##### **I: Prosecution History.**

The application as filed contained 16 claims. In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In an Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In an amendment dated November 26, 1997, the Applicants canceled claims 6, 13, and 17; amended claims 1, 3-5, 7, 11, 18, and 20; and added new claims 26-38. In an amendment dated July 23, 1998, the Applicants amend claims 1, 3-5, 7, 18-19, 26-33, and

36-37; and add new claims 39-44. Thereafter, the Patent Office suspended prosecution of the application for approximately 18 months because "a reference relevant to the examination of this application may soon become available."

At the time of issuance of the outstanding Office action, claims 1, 3-5, 7, 11, and 18-44 were pending. In an interview on June 22, 2000, the Examiner requested submission of a renumbered claim set. Thus, the pending claims have been canceled and new claims 45-79 have been substituted therefor. A table correlating old and new claims is set forth for the Examiner's convenience.

Current Claim	Corresponding Old Claim	Comments
Claim 45.	Claim 18.	
Claim 46.	Claim 19.	
Claim 47.	Claim 20.	
Claim 48.	Claim 21.	
Claim 49.	Claim 22.	
Claim 50.	Claim 32.	
Claim 51.	Claim 11.	
Claim 52.	Claim 23.	
Claim 53.	Claim 30.	
Claim 54.	Claim 41.	
Claim 55.	Claim 31.	
Claim 56.	Claim 24.	
Claim 57.	Claim 25.	
Claim 58.	Claim 38.	
Claim 59.	Claim 39.	
Claim 60.	Claim 43.	
Claim 61.	Claim 19.	
Claim 62.	Claim 44.	
Claim 63.	Claim 34.	
Claim 64.	Claim 35.	

Claim 65.	Claim 36.	
Claim 66.	Claim 1.	Additional limitations specifying human polynucleotide and 23 kD polypeptide
Claim 67.	Claim 26.	
Claim 68.	Claim 29.	
Claim 69.	Claim 3.	Additional limitation specifying 23 kD polypeptide
Claim 70.	Claim 4.	
Claim 71.	Claim 5.	
Claim 72.	Claim 40.	
Claim 73.	Claim 7.	
Claim 74.	Claim 42.	Additional limitation specifying human polypeptide
Claim 75.	Claim 37.	

The suspension of this application for more than a year has been detrimental to the Applicants' continued commercial development of this technology, and the Applicants are quite interested in expeditious allowance, now that prosecution has resumed and the "relevant reference" has become available. The claim amendments herein are solely for the purpose of clarity and expediting allowance, and are unnecessary to overcome the Patent Office's rejections. The Applicants reserve the right to pursue the subject matter of claims as originally filed (or later introduced) in subsequent applications, such as continuing applications.

**II. The Patent Office's rejection of claims 1, 37, and 42 under 35 U.S.C. §112, first paragraph, for lack of written descriptive support should be withdrawn.**

In paragraph 6 of the Office action, the Patent Office rejected claims 1, 37, and 42 under 35 U.S.C. 112, first paragraph, alleging that these claims contain subject matter "which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was; filed, had possession of the claimed invention." Claim 37 was rejected due to its dependence from claim 1. New claims 66 and 74 are analogous to rejected claims 1 and 42.



Although the rejection spans approximately three pages, the Patent Office's principal objections appear to be that the breadth of the claims is such that the claims encompass polynucleotides from other species and polynucleotides which encode variant polypeptides so long as receptor binding activity is maintained. The Patent Office acknowledges the presence of Examples relating to a human Flt4 ligand, and there are no impediments to identifying other human molecules that may be allelic variants, for example. Solely to expedite allowance,<sup>1</sup> the Applicants have included a limitation in claims 66 and 74 to specify a human polynucleotide sequence. The Applicants' teachings of a human sequence, combined with their many additional teachings related to VEGF-C processing, Flt4 binding, Flt4 binding assays, hybridization techniques, and the like, conveys possession of the human genus recited in the claims to a person of ordinary skill. These amendments render moot the rejection for lack of written description, and the rejection should be withdrawn.

**III. The Patent Office's rejection of claims 1, 3-5, 7, 11, and 18-44 under 35 U.S.C. §102(e) should be withdrawn.**

In paragraph 8 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 11, and 18-44 under 35 U.S.C. §102(e), as allegedly being anticipated by Hu et al. (U.S. Pat. No. 5,935,820). The rejection specifically addressed only claims 11 and 18, although it contained some general reasoning apparently directed at the other claims:

Hu et al. disclose a polynucleotide, SEQ ID NO:1, which encodes a polypeptide, SEQ ID NO:2, which includes a domain defined by 8 cysteine residues of the VEGF family, and which is capable of binding to human Flt4 receptor tyrosine kinase. The instant claims indicate that the polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein, however, this limitation only further defines the processed protein and places no material limitations on the polynucleotide. Claim 11 further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide. Claim 18 is directed to a polynucleotide which lacks a

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<sup>1</sup> The Applicants reserve the right to dispute the factual and legal premises upon which the rejection is based, and pursue claims of the original or greater scope in continuing applications.

The Applicants also observe that the Hu et al. '820 patent cited in paragraph 7 teaches only a single polynucleotide species yet purports to claim a genus using hybridization claim limitations. See, e.g., Hu et al. claims 41 and 50. The Examiner is requested to clarify the Patent Office's position as to when a single polynucleotide species provides a written description of a hybridization genus that satisfies §112, first paragraph.

portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a "polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" anticipates this claim.

(Office action at p.6.)

The Applicants respectfully traverse.

At the outset, the Applicants wish to clarify certain factual and legal issues raised by the above-quoted rejection. First, the rejection is factually incorrect in that Hu et al. neither discloses nor suggests that any polypeptide binds actually binds to Flt4. In fact, Hu et al. makes no mention of the Flt4 receptor whatsoever, and fails to identify any receptor for "VEGF2" whatsoever. Second, the Applicants object to the Patent Office's suggestion that the scope or wording of the claims of the Hu et al. patent have any relevance to whether Hu et al. is anticipatory under §102(e). The application that matured into the Hu et al. patent was filed on March 27, 1997, more than two years after the filing date of the present application, and after the publication of a PCT application based on the present application (See WO 97/05250, published February 13, 1997), and after the publication of the present inventors own work in prominent scientific journals that would have come to the attention of Hu et al.<sup>2</sup> Still more of the present inventor's publications were available to Hu et al. in 1997-1999, during prosecution of the Hu et al. application. (See, e.g., Joukov et al., "Proteolytic Processing regulates receptor specificity and activity of VEGF-C," *EMBO J.* 16(13): 3898-3911 (1997).) The relevant inquiry under §102(e) is the inquiry of what was "described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent." This inquiry requires the Patent Office to ignore what was claimed in the Hu et al. patent, which may have been tainted by knowledge of the present invention, as explained above. The relevant inquiry must focus on what was described in those Hu et al. priority applications that have a filing date that could have

<sup>2</sup> See, e.g., Joukov et al., "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.* 15(2): 290-298 (1996); and Kukk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V, Alitalo K., "VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development," *Development*, 122(12): 3829-37 (1996).

preceded the invention date of the applicants.<sup>3</sup> See, e.g., *In re Benno*, 226 USPQ 683, 686 (Fed. Cir. 1985) ("The scope of a patent's claims determines what infringes the patent; it is no measure of what it discloses. A patent discloses only that which it describes....") The limited teachings of Hu et al. are discussed below.

The Applicants also dispute that the Patent Office's reasoning, even if correct, supports a legitimate anticipation rejection. Independent claims 1, 3, 7, and 42 (now claims 66, 69, 72, and 74) are all directed to host cells that have been transformed or transfected with a nucleic acid and that express an approximately 23 kD polypeptide encoded by the nucleic acid that has particular structural and functional characteristics, such as a particular size or sequence and/or the ability to bind the Flt4 receptor. The Hu et al. patent neither discloses nor suggests the recited polypeptides, or host cells that make such polypeptides, or the activities of the polypeptides. (There is no description in Hu et al. of a 23 kD Flt4 ligand polypeptide or of a host cell that produces such a polypeptide.)

Notwithstanding these claim limitations, the Patent Office rejected the claims. Under the Patent Office's analysis, "this limitation only further defines the processed protein and places no material limitations on the polynucleotide." The Patent Office has apparently ignored the fact that these particular claims are not directed to an isolated polynucleotide, but rather to a host cell that produces a polypeptide having certain characteristics. The Patent Office has failed to explain why a limitation on a novel and nonobvious protein produced by a host cell is insufficient to render novel a claim to the host cell that produces the protein. The Patent Office has apparently ignored the axiom that anticipation of a claim under §102 can be found only if the prior art discloses *every element* of the claim. See, e.g., *In re King*, 801 F.2d 1324, 1326 (Fed. Cir. 1986). An anticipation of a recombinant, protein-producing host cell claim does not exist merely because a polynucleotide has allegedly been described in the prior art.

The Patent Office rejected claim 18 (now claim 45), directed to a polynucleotide, on the basis that "Claim 18 is directed to a polynucleotide which lacks a portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor

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<sup>3</sup> The Applicants reserve the right to dispute whether Hu et al. qualifies as a §102(e) reference, on the grounds that Hu et al. is not a patent granted on an application filed before the invention thereof by the applicant.

tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a 'polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2' anticipates this claim." This reasoning is based on an improper focus on what the cited patent *claimed*, rather than what it *described*. See *In re Benno, supra*. If one reads the Hu et al. patent to determine what Hu et al. actually *describes* as "a polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" one finds descriptions such as the following:

The polynucleotide of this invention . . . contains an open reading frame encoding a protein of about 350 amino acid residues of which approximately the first 24 amino acid residues are likely to be leader sequence such that the mature protein comprises 326 amino acids.

(Hu et al. at Col. 3, lines 56-63.)

Thus, Hu et al. describes a polynucleotide that encodes the "mature portion of a protein consisting of SEQ ID NO: 2" as a polynucleotide that comprises the final 326 codons of SEQ ID NO: 2.<sup>4</sup> A study of the approximately 350 codon sequence in Hu et al. (Figures 1-2; SEQ ID NO: 2) shows that the mature protein of 326 amino acids includes the carboxy-terminal domain Balbiani Ring 3 Protein cysteine motifs. Because the "mature protein" described in Hu et al. includes the BR3P domain and falls outside the scope of claim 45, claim 45 is not anticipated. Claim 11 (now 51), which depends indirectly from claim 45, also is not anticipated.

In paragraph 9, the Patent Office acknowledged that certain subject matter was free of the prior art, and in paragraph 8, it suggested that claim 11 might have been an attempt to claim that allowable subject matter, except that its limitation "further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide." Claim 11 (now claim 51) is patentable over the art because it depends from claim 45, as explained above. However, the Applicants wish to direct the Patent Office's attention to claim 60 (formerly 43), which claims a nucleic acid and contains the explicit limitation "said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125." The Applicants respectfully submit that claim 60 satisfies the Patent

<sup>4</sup> The Applicants reserve the right to present evidence that the alleged signal peptide taught in Hu et al. does not operate as a signal peptide at all.

Office's own criteria for allowable subject matter in this case, and should not have been rejected at all.

For the foregoing reasons, the Hu et al. patents neither disclose nor suggest the claimed invention, and the rejections based on Hu et al. under 35 U.S.C. §102(e) should be withdrawn.

#### IV. Interview Follow-up

During the interview with Examiners Saoud and Kunz and the Applicants' attorneys, the Examiners acknowledged that host cells which produced a fully processed VEGF-C polypeptide of approximately 23 kD were novel and unobvious over the two Hu et al. patents of record, and raised the question of whether this result of the Applicants' was due uniquely to the host cell chosen. The Applicants have filed herewith a declaration to provide evidence that they have succeeded in producing a Flt4 ligand of approximately 23 kD in several other host cells. An executed version of the declaration will be submitted under separate cover.

#### V. Information Disclosure

On March 21, 2000, the Patent Office issued a third patent to Hu et al., U.S. patent No. 6,040,157. The '157 patent is a CIP that was filed in December, 1997, after many publications by the present applicants and after the present application was filed. The Applicants wish to draw the Examiner's attention to the '157 patent. For §102(e) purposes, the '157 patent is cumulative to the '540 and '820 patents of record. (To the extent it is not cumulative in disclosure, it is not citable as *prima facie* prior art, because the non-cumulative disclosure is not entitled a date that precedes the January 12, 1996, filing date of the present application.)

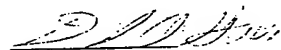
VI. Summary

The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

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Dated: August 4, 2000

  
David A. Gass  
Registration No. 38,153



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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al. ) Title: RECEPTOR LIGAND  
Serial No: 08/585,895 ) Group Art Unit: 1646  
Filed: January 12, 1996 ) Examiner: Christine Saoud

TRANSMITTAL LETTER

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith is an executed declaration of Kari Alitalo for entry into the file for the above-identified matter. An unsigned version of this declaration was previously filed, together with an amendment, on August 4, 2000. The Applicants believe that this declaration should be entered without petition or fee for additional extension of time, because a fully responsive amendment to the Office action of April 4, 2000, has already been filed. However, if extension of time is required, please consider this transmittal to be a request therefor, and charge an additional extension fee to deposit account No. 13-2855.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 10, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al. ) Title: RECEPTOR LIGAND  
Serial No: 08/585,895 ) Group Art Unit: 1646  
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David A. Gass





#34  
12.7

**PATENT**

Attorney Docket No. 28967/33072  
LUD 5453.1

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In the Application of: Alitalo et al.

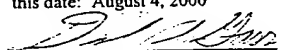
Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: SAOUD, Christine

) I hereby certify that this paper and the  
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) for Patents, Washington, DC 20231, on  
) this date: August 4, 2000  
)   
) David A. Gass  
) Reg. No.: 38,153  
) Attorney for Applicants  
)

**Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo**

I, Dr. Kari Alitalo, declare and state as follows:

**Introduction**

1. I am a co-inventor of the subject matter of the above-identified patent application (hereinafter "the patent application"). I make this declaration to provide evidence to the Patent Office that may be relevant to the patentability of pending claims. Specifically, some of the pending claims in the application relate to recombinant host cells that produce a mature Flt4 receptor ligand polypeptide of approximately 23 kD (as assessed by SDS-PAGE under reducing conditions). This declaration is intended to provide evidence and confirmation that we have achieved expression of this mature form using a variety of host cells transformed/transfected with a full length cDNA encoding a 419 residue prepro-form of the ligand.

## Evidence

### I. Introduction

2. My co-inventor Dr. Joukov and I (together with others in my laboratory) have conducted substantial experiments to evaluate the proteolytic processing of human prepro-VEGF-C, a protein of 419 amino acids, into a mature form which has undergone substantial N-terminal and C-terminal processing. The results of many of the VEGF-C processing experiments are succinctly and accurately reported in our publication Joukov *et al.*, "Proteolytic processing regulates receptor specificity and activity of VEGF-C," *EMBO J.* 16(13): 3898-3911 (1997). I hereby reaffirm the accuracy of the data reported in that paper, which I incorporate by reference, and summarize only briefly in the next paragraphs of the introduction.

3. The VEGF-C gene encodes a mRNA for the synthesis of a prepro-protein F114 ligand precursor of 419 amino acids. (The complete 419 codon cDNA was deposited with the ATCC and is cross-referenced at pages 6, 28-29, and 39 of the patent application, and its sequence is deposited as SEQ ID NOs: 44 and 45 in the Sequence Listing.) The "pre-pro-protein" is processed to remove a signal peptide and two pro-peptides to produce a fully mature, most active form of VEGF-C. Initially, the "pre" part or signal sequence is cleaved off upon its translocation through the cellular membrane in the rough endoplasmic reticulum of the synthesizing cells. The rest of the polypeptide is then translocated across the cell membrane upon its continued elongation synthesis.

4. A proteolytic cleavage to cleave a C-terminal pro-peptide occurs preferentially between amino acid residues 227 and 228, separating the N-terminal and C-terminal halves (roughly) of the constituent polypeptides.<sup>1</sup> This polypeptide, once cleaved in the middle, is

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<sup>1</sup> The C-terminal half contains cysteine residue repeat patterns reminiscent of the Balbiani Ring 3 Protein (BR3P). The N-terminal half contains a series of cysteine residues in a pattern shared with other members of VEGF/PDGF family.

Position 227 of the 419 codon sequence corresponds to position 125 of SEQ ID NO: 33, the sequence referred to in the claims of the patent application.

only partially active relative to the fully mature, proteolytically processed VEGF-C that is created upon removal of the N-terminal pro-peptide from the N-terminal half.

5. Another cleavage of the VEGF-C protein precursor to remove an N-terminal pro-peptide occurs on the amino-terminal side of the domain that shares a cysteine motif in common with other members of the VEGF-PDGF family. In our experiments we have observed that this second pro-peptide cleavage occurs in at least two preferential peptide bonds: one is between amino acid residues 102 and 103,<sup>2</sup> and the other one between residues 111 and 112. These two alternative cleavages that remove the N-terminal pro-peptide produce fully processed forms of the Flt4 ligand which have a similar potency of high affinity binding to KDR/VEGFR-2, which is expressed in blood vascular endothelium and lymphatic endothelium and to Flt4/VEGFR-3, which is predominantly expressed in the lymphatic endothelium. The fully processed mature forms of VEGF-C that are most active have a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

## II. Recombinant Host cells that produce a fully processed VEGF-C of about 23 kD

6. Several of our initial experiments were done using 293EBNA or 293 T-cells as host cell systems for the transfection of VEGF-C expression vectors. These cells were fairly efficient in the processing of the prepro-VEGF-C into the fully processed ~23 kD form. In the initial experiments described in our patent application, VEGF-C was eluted from

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<sup>2</sup> Position 103 (Threonine) of the 419 codon sequence corresponds with position 1 of SEQ ID NO: 33, the sequence referred to in the claims of the patent application.

an Flt4-EC affinity matrix using pH 2.4, which seemed to enhance (but was not necessary for) the proteolytic processing into the mature form.<sup>3</sup>

7. We have also observed similar proteolytic processing in COS monkey cells and in the HT1080 human fibrosarcoma cells. Experimental details with COS and HT1080 cells are reported in Joukov *et al.* (1997), *supra*. The cells were grown in a conventional commercial media (Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum.<sup>4</sup> The vector used for transformation of these cells was a pREP7 vector containing the 419 codon VEGF-C cDNA, essentially as described in the patent application. Processing to produce the ~ 23 kD form was observed in both COS and HT1080 cells, although it was significantly less efficient than in the 293 cells.

8. We have also used the MCF-7 breast carcinoma cell line to produce the ~23 kD mature form of VEGF-C. Unlike the 293 or COS cells, MCF-7 cells do not replicate the transfected plasmid. MCF-7 cells were transfected with pEBS7 expression vector<sup>5</sup> containing a cDNA insert coding for human prepro-VEGF-C cDNA (419 codon form) or with an empty vector, and stable cell pools were selected. The transfected MCF-7 cells were grown in RPMI-1640 medium (a commercially available medium) containing 10% FCS and 150 µg/ml Hygromycin B. To study forms of VEGF-C produced, the cells were metabolically labeled,

<sup>3</sup> Like all enzymatic reactions, the proteolytic cleavages in the VEGF-C polypeptide backbone occur in an enzyme- and substrate concentration- dependent manner, and can be influenced by factors such as pH, time, and protease inhibitors. Our data suggests that the enzymes that cleave the N-terminal pro-peptide from pro-VEGF-C are secreted from both VEGF-C-producing and from other cell types. We also have observed increased processing upon the depletion of the culture medium of fetal calf serum.

<sup>4</sup> By way of comparison, Example 13 of the patent application describes culturing 293-EBNA cells in DMEM-0.2% BSA. As explained in the previous footnote, lower serum levels in the media appears to correlate with increased processing.

<sup>5</sup> The pEBS7 expression vector was known in the literature at least as early as 1991, years before the priority date of the application. (See, e.g., Peterson, C. and Legerski, R., "High-frequency transfection of human repair-deficient cell lines by an Epstein Barr virus-based cDNA expression vector," *Gene*, 107(2): 279-284 (1991)). We selected pEBS7 because this vector promotes high expression levels in MCF-7 cells. The vector contains a CMV promoter and hygromycin B and ampicillin resistance genes.

and after 96 hours, VEGF-C was bound to an Flt4 affinity substrate.<sup>6</sup> The bound proteins were analyzed in 12.5% SDS-PAGE under reducing conditions. As can be seen from Figure A, the VEGF-C in the culture medium that bound to the soluble receptor Flt4-Ig affinity substrate consists of both the 29/31 kD and ~23 kD forms. Thus, expression of VEGF-C protein in these cells occurs in smaller quantities than other cells we have tested which replicate the transfected plasmid, yet we can recover both the 31/29 and ~23 kD forms of VEGF-C from the culture medium of these cells using the type of affinity matrix described in the patent application.

9. We also have achieved production of the ~23 kD-form of VEGF-C using the baculovirus expression system in insect cells. In one set of the experiments, 3 million Sf-9 cells each were infected with baculoviral clones 32/1-32/5 and 34/1-34/5 expressing full length (419 codon) untagged hVEGF-C under the polyhedrin promoter.<sup>7</sup> Seven days post-infection, the supernatant was harvested and the remaining cells lysed in 350 µl RIPA buffer. Ten microliters of supernatant of clone 32/1 and 5 µl lysate of clones 32/1 - 32/5 and 34/1 - 34/5 were subjected to 15% SDS PAGE, and VEGF-C specific bands were immunodetected after Western blotting using antiserum raised against VEGF-C peptide (residues 104-120, or

<sup>6</sup> The affinity substrate that we used was Flt4(1-3)Fc, which comprises the three immunoglobulin-like regions of the Flt4 extracellular domain which have been shown to be responsible for ligand binding. Thus, this affinity matrix is the functional equivalent of the Flt4-EC affinity matrix described in the patent application.

<sup>7</sup> The cell line Sf9 is a clonal isolate of Sf21 cells, which are derived from ovary cells of the fall army worm, *Spodoptera frugiperda*. The cell line was maintained as adherent culture at 27°C in TMN-FH media completed with fetal bovine serum to a final concentration of 10%. In addition, 100 mg/ml streptomycin and 10 units/ml penicillin were used to minimize the risk of bacterial contamination. The cells were cultured using routine and standard procedures for these experiments. (See, e.g., O'Reilly et al., *Baculovirus Expression Vectors: a laboratory manual*. W.H. Freeman and Company, New York, 1992: pp. 109-122).

For virus production and amplification, 5 baculoviral clones (1-5) were purified from two transfection supernatants (32 and 34), that were obtained using the FASTBAC system (GIBCO/Life Technologies) according to the instructions of the manufacturer. Transfections 32 and 34 were performed using two bacmid DNA preparations from independently obtained clones using shuttle vector pFB1-hVEGF-C-FL. Stock virus was obtained by two rounds of amplification after plaque purification. For the first amplification, 2.5 Mio. Sf-9 cells were inoculated with the whole purified plaque and incubated for 5 days. For the second amplification 8 Mio. Sf-9 cells were inoculated with 1/40 of the total virus obtained in the first amplification step and incubated for 5 days.

2-18 of the mature form). The results, depicted in Figure B, show clearly that the major form of VEGF-C in the lysates of 7 day p.i. cells is the 21/23 kD form. Uncleaved (prominent band), 29/31 kDa (prominent band), and ~23 kDa forms (weaker band) were present in the supernatant. These experiments show that insect cells also cleave the VEGF-C protein to the ~23 kD mature form and that this insect cell expression system can provide a source for large-scale production of the ~23 kD form of the protein.

10. In another series of experiments, we transfected the MeWo cell line,<sup>8</sup> established from a lymph node metastasis of a nodular malignant melanoma, to constitutively overexpress a prepro-VEGF-C cDNA (419 codon form)<sup>9</sup>. Ordinary commercial media was employed for these experiments also (RPMI 1640 medium with 5% fetal bovine serum (FBS), purchased from Gibco BRL, Grand Island, NY). As determined by Northern analysis, the parental MeWo cell line and three vector-transfected control clones (MeWo/control) did not express any detectable amounts of VEGF-C mRNA *in vitro* or *in vivo*. Three VEGF-C transfected cell clones (MeWo/VEGF-C) expressed high levels of VEGF-C mRNA in culture, as well as in tumors (when introduced into mice) that reached the size of ~1200 mm<sup>3</sup>. Western blot analyses using antibodies raised against a VEGF-C peptide confirmed that high VEGF-C mRNA levels correlated with high amounts of VEGF-C protein expression. We

<sup>8</sup> The human malignant melanoma cell line MeWo (Sordat, B.C. M., Y. Ueyama, and J. Fogh. 1982. Metastases of tumor xenografts in the nude mouse. In *The nude mouse in experimental and clinical research*. J. Fogh, and B.C. Giovanella, editors. Academic Press, New York. 95-147; Kerbel, R.S., M.S. Man, and D. Dexter. 1984. A model of human cancer metastasis: extensive spontaneous and artificial metastasis of a human pigmented melanoma and derived variant sublines in nude mice. *J Natl Cancer Inst.* 72:93-108), kindly provided by Dr. Robert S. Kerbel (Sunnybrook Health Science Centre, Toronto, Canada)

<sup>9</sup> A 1997 bp full-length (419 codon) human VEGF-C cDNA (GenBank accession number X94216) was cloned into a pcDNA3.1/Zeo expression vector (Invitrogen, San Diego, CA) which contains a CMV-enhancer-promoter and a Zeocin selection cassette. The sequence and the orientation of the VEGF-C gene in the construct were verified by restriction mapping and by direct sequencing using the Sanger dideoxy method. Subconfluent cell cultures were transfected either with pcDNA3.1/Zeo vector containing the full-length human VEGF-C cDNA in sense orientation or with the vector alone using the Superfect transfection reagent (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were split 1:5 into their full growth medium containing 50 mg/ml Zeocin (Invitrogen) to select transfectants. Stably transfected cell clones were individually expanded and analyzed for VEGF-C mRNA expression and protein secretion.

detected a strong band of approximately 60 kDa in cell lysates of VEGF-C transfectants, corresponding to VEGF-C precursor, and only trace amounts in control cells. Large amounts of the secreted 31 kDa form were observed in culture supernatants of VEGF-C transfected clones, whereas the secreted protein was not detectable in supernatants of control cells. The mature ~23 kDa VEGF-C form was detected in tumor lysates.

11. The foregoing experiments demonstrate that we were able to recombinantly express the ~23 kD mature form of the Flt4 ligand VEGF-C in a variety of human cell lines transfected with a 419 codon prepro-VEGF-C cDNA, as well as in a Cos monkey cell line and an insect cell line.

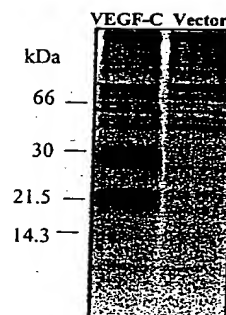
#### Certification

12. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

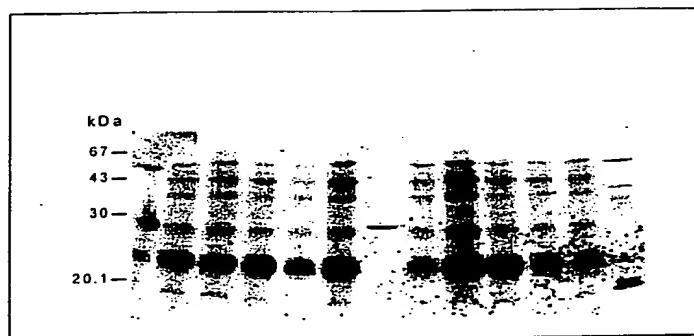
\_\_\_\_\_  
Kari Alitalo

Date: \_\_\_\_\_

**Figure A**



**Figure B**





PATENT

Attorney Docket No.: 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al. ) I hereby certify that this paper is being  
Serial No: 08/585,895 ) deposited with the United States Postal  
Filed: January 12, 1996 ) Service, in an envelope addressed to the:  
Title: RECEPTOR LIGAND ) Commissioner for Patents, Box Issue  
Allowed: October 24, 2000 ) Fee, Washington, D.C. 20231, utilizing  
Batch No.: U18 ) the "Express Mail Post Office" under  
Group Art Unit: 1647 ) Mailing Label No. EL566464161US on  
Examiner: C. Saoud ) this date:  
January 24, 2001



*Suzarah A. Maguigad*  
Suzarah A. Maguigad

AMENDMENT AFTER ALLOWANCE PURSUANT 37 C.F.R. § 1.312

Commissioner for Patents  
Box Issue Fee  
Washington, D.C. 20231

Dear Sir:

Please amend this application as follows:

AMENDMENTS

In the Specification:

At page 1, line 3, after "August 1, 1995.", please delete the following priority claim, which was introduced by way of Amendment filed July 23, 1998:

"This application is also a continuation-in-part of U.S. Patent Application Serial No.

08/340,011, filed November 14, 1994, now U.S. Patent No. 5,776,755."

At page 8, line 7, please delete "Figure 2 schematically depicts" and insert --  
Figures 2A and 2B schematically depict--.

At page 8, line 29, please delete "Figure 9B shows" and insert --Figures 9B-D  
show--.

At page 8, line 30, please delete "Figures 10A-10B" which was introduced by  
way of Amendment filed November 26, 1997 and insert --Figures 10A-D--.

At page 14, line 32, please delete "Figure 2" and insert --Figures 2A and 2B--.

At page 27, line 30, please delete "Figure 9B" and insert --Figures 9B through  
9D--.

At page 28, line 1, please delete "Figure 10" and insert --Figures 10A through  
10D--.

At page 28, line 6, please delete "Fig. 9B" and insert --Figures 9B through  
9D--.

At page 29, line 13, please delete "Fig. 10" and insert --Figures 10B and  
10C--.

#### **REMARKS**

Applicants request entry of the foregoing amendments, which relate solely to  
formal matters. These amendments are being presented prior to or concurrently with payment  
of the issue fee as required by Rule 312. The amendments do not affect the scope or content of  
the allowed claims. The Patent Office is authorized to charge any fee associated with this  
amendment to Deposit Account No. 13-2855.

The amendment to page 1 amounts to a cancellation of a priority claim to an

application that was filed in November, 1994. The Applicants continue to maintain their priority claim to U.S.S.N. 08/510,133, filed August 1, 1995, as stated in the application as originally filed. The sole purpose behind cancellation of the 1994 priority claim is to maximize patent term of the eventual patent, because it is the Applicants' understanding of current law that the term of this patent will be measured from the earliest claimed priority date. The priority claim cancellation is not intended as an admission of whether or not the claimed invention would be entitled to priority, if the priority claim to the November, 1994 application were maintained. The Applicants reserve the right to maintain the same priority claim for subject matter that may be pursued in related applications, such as continuations, continuations-in-part, divisional applications, reissue applications, or the like. It is the Applicants' understanding from prosecution that the subject matter of the allowed claims has been deemed patentably distinct from any subject matter disclosed in art of record, including subject matter disclosed in U.S. patent issued to Human Genome Sciences (Hu et al., U.S. Patent No. 5,935,820) that was considered by the Examiner. (This patent was cited by the Examiner as a reference under §102(e) and distinguished by the Applicants. See Amendment dated August 4, 2000, at pages 11-15.) Thus, the presence or absence of the priority claim raises no patentability issues.<sup>1</sup>

The remaining amendments to the specification merely conform the specification to the formal drawings submitted concurrently herewith. Figures 2, 5, 9 and 10 were prepared

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<sup>1</sup> The November, 1994 patent application has issued as U.S. Patent No. 5,776,755. The '755 patent is not prior art under §102(e) because, to the extent the '755 patent discloses or suggests the present invention, the relevant disclosure is a disclosure of the present inventors' own work. Because the relevant portions of the '755 patent constitute the inventor's own work, the relevant filing date of the '755 patent was not "before the invention thereof by the applicant" as required by §102(e). (It is impossible to disclose the inventors' own work before the inventors invented it.)

on multiple sheets and/or renumbered in order to comply with the Draftsman's requirements. The specification has been amended to reflect the fact that these figures will be multiple pages in the issued patent.

These amendment add no new matter and do not raise any new patentability issues that would require any substantive examination by the Examiner.

In view of the foregoing, the applicant respectfully requests the granting of the amendment after allowance.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,  
MURRAY & BORUN

By:



David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606

January 24, 2001

Allowed: October 24, 2000  
Batch No.: U18  
Application No.: 08/585,895

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.  
☒ Small entity status has been established and is still effective.  
☐ Has not been established.

2. Deposit Account and Refund Authorization

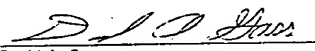
- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.  
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
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January 24, 2001

By:

  
David A. Gass  
Reg. No: 38,153



Allowed: October 24, 2000  
Batch No.: U18  
Application No.: 08/585,895

**PATENT**  
**28967/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Alitalo et al.	) Allowed: October 24, 2000
	) Batch No.: U18
Serial No: 08/585,895	) Application No.: 08/585,895
	)
Filed: January 12, 1996	) Title: RECEPTOR LIGAND
	)
	) Group Art Unit: 1647
	)
	) Examiner: C. Saoud

**TRANSMITTAL LETTER**

*Commissioner for Patents*  
*Washington, D.C. 20231*

Sir:

Transmitted herewith are the following for entry in the above-identified case:

1. Amendment After Allowance;
2. Thirty sheets of formal drawings (Figs. 1, 2A-2B, 3-4, 5A-5C, 6-8, 9A-9D, 10A-10D, 11-12, 13A-13B, 14A-14B, 15A-15B, 16A-16B, 17-18); and
3. Request for correction of Drawing with sketch showing proposed change to Figure.

**CERTIFICATE OF MAILING (37 CFR 1.8)**

I hereby certify that this paper is being deposited with the United States Postal Service, in an envelope addressed to the: Commissioner for Patents, Box Issue Fee, Washington, D.C. 20231, utilizing the "Express Mail Post Office" under Mailing Label No. EL566464161US on January 24, 2001.

*Suzanne A. Maguigad*  
Suzanne A. Maguigad

Allowed: October 24, 2000  
Batch No.: U18  
Application No.: 08/585,895

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.  
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☐ Has not been established.

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- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.  
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
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(312) 474-6300

January 24, 2001

By: 

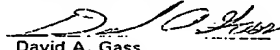
David A. Gass  
Reg. No: 38,153

Nov. 2. 2000 3:40PM MARSHALL, O'TOOLE

No. 5291 P. 2/2  
From: 0819

**PATENT**  
**28967/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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Filed: January 12, 1996	)	date: Date: November 2, 2000.
	)	Fax No.: (703) 305-3014
Title: Receptor Ligand	)	
	)	
Group Art Unit: 1646	)	David A. Gass
	)	Registration No. 38,153
Examiner: Christine Saoud	)	Attorney for Applicants
	)	

Commissioner for Patents  
Washington, D.C. 20231

**CHANGE OF ADDRESS**

Sir:

The undersigned is an attorney of record in this case. Please mail all correspondence in this case to the undersigned at the address below :

David A. Gass  
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The attorney's phone number is (312) 474-6300.

Respectfully submitted,

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Dated: November 2, 2000

  
David A. Gass  
Registration No. 38,153

**OK to Enter**





Issue Date: October 24, 2000

Issue Batch No.: U18

Application No.: 08/585,895

PATENT

Attorney Docket No.: 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Allowed: October 24, 2000

Batch No.: U18

Group Art Unit: 1647

Examiner: C. Saoud

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) Label No. EL566464161US on this date:

) January 24, 2001

) *Suzanne A. Maguigad*  
) Suzanne A. Maguigad

REQUEST FOR APPROVAL OF DRAWING CHANGES

Commissioner for Patents  
Washington, D.C. 20231  
Attn: Official Draftsperson

Dear Sir:

AMENDMENT

Applicants hereby request approval of the drawing changes as shown in red ink on the attached copy of the informal drawing (FIG. 9B) for the above-identified application. Support for the requested change may be found throughout the specification as originally filed as explained below. No new matter has been added. The requested change is embodied in the formal drawings filed herewith. The Patent Office is authorized to charge any fee required in connection with the filing of this request to Deposit Account 13-2855.

#1  
Bg  
3/5

#### REMARKS

FIG. 9B illustrates the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA, in which the cleavage site for the putative signal peptide is indicated with a shaded triangle, as disclosed in the specification at page 8, lines 29-31.

The drawing change is being made solely to correct the location of the shaded triangle which indicates the cleavage site demarking a mature VEGF-C protein. Particularly, the shaded triangle should be positioned between "Arg" and "Thr" and not "Ser and Arg". The position of the shaded triangle indicates the start of the designation of the portions of SEQ ID NO: 33 which correspond to the "mature" forms of VEGF-C. The change finds support as originally filed because the description of the amino terminus of a mature form of VEGF-C is found in the specification at p. 23, lines 5-10, and is confirmed at page 25, line 27 to page 26, line 6 (from which it is apparent that the first 13 amino acid residues of a secreted Flt4 ligand are encoded by the thirty-nine 3' bases of SEQ ID NO: 25 that begin ACAGAAGAGACT...). Similar changes to the numbering of residues in the Sequence listing were made in an Amendment filed by the Applicants on November 26, 1997, and were approved by the Examiner. The changes made herein are consistent with what was earlier done the prosecution of this application.

In view of the foregoing, it is submitted that the change to FIG. 9B does not introduce new matter into the disclosure of the application or to the drawings. Accordingly, applicants request approval of the above drawing change.

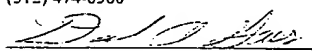
Corrected formal drawings will be provided for the above-identified application.

Respectfully submitted,

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MURRAY & BORUN  
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Chicago, Illinois 60606-6402  
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January 24, 2001

By:

  
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APPROVED:   
 DRAFTSMAN:   
 FIG.   
 CLASSIFICATION:

1/30

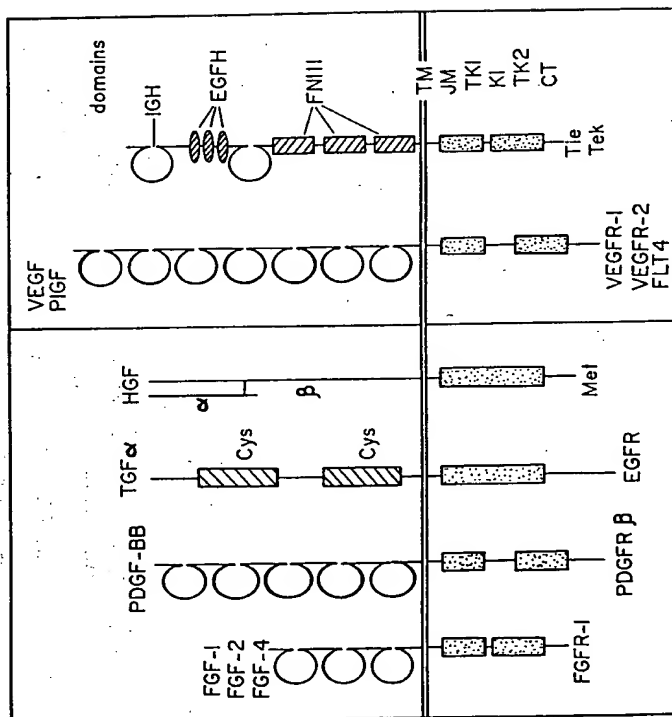
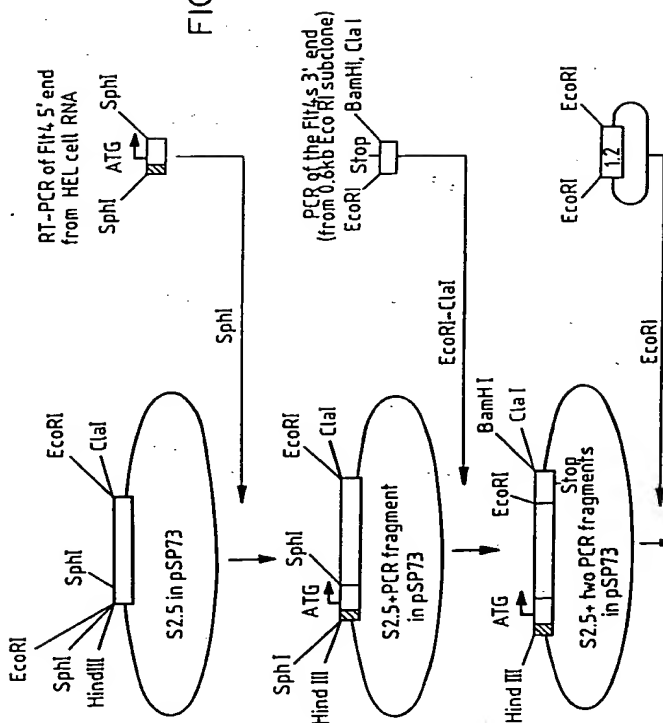


FIGURE 1

FIGURE 2A

2 / 30





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Washington, D.C. 20231

FROM: George M. Rayford, Manager  
Correspondence & Mail Division

SUBJECT: Receipt of Papers and Fees File Under 37 CFR 1.10 By  
Express Mail

The filing date of \_\_\_\_\_ is the correct date. The date on the Express Mail label under 37 CFR 1.10 is \_\_\_\_\_. On that date the PTO was closed all day due to \_\_\_\_\_ adverse weather conditions (authorized by Office of Personnel Management) or a \_\_\_\_\_ normally scheduled Federal holiday within the District of Columbia. In accordance with 37 CFR 1.6 the papers have been stamped with the next succeeding day which is not a Saturday, Sunday or Federal holiday within the District of Columbia. The provision of 35 U.S.C. 21 (b) apply.

The papers were not stamped with the date on the certificate of mailing by Express Mail because the date on the certificate does not coincide with the date of deposit on the Express Mail label which the PTO takes as evidence of when the package was mailed.

Date on certificate of mailing by Express Mail is 1-12-96

Date on Express Mail label is 1-12-96

Date of receipt in PTO is 1-16-96

Therefore, the filing date is 1-16-96

The papers are not entitled to the benefits of 37 CFR 1.10 because:

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SIGNED: Julia A. Woodward

DATE: 1-19-96

PAGE: 1

**SEQUENCE VERIFICATION REPORT**  
**PATENT APPLICATION US/08/585,895**

DATE: 04/11/96  
TIME: 14:24:34

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Line

Error

Original Text



PAGE: 5

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PATENT APPLICATION US/08/585,895

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206 (1) SEQUENCE CHARACTERISTICS:  
207 (A) LENGTH: 20 base pairs  
208 (B) TYPE: nucleic acid  
209 (C) STRANDEDNESS: single  
210 (D) TOPOLOGY: linear  
211  
212 (ii) MOLECULE TYPE: DNA (genomic)  
213  
214 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
215  
216 GTTGCCTGTG ATGTGCACCA 20  
217  
218 (2) INFORMATION FOR SEQ ID NO:13:  
219  
220 (1) SEQUENCE CHARACTERISTICS:  
221 (A) LENGTH: 18 amino acids  
222 (B) TYPE: amino acid  
223 (C) STRANDEDNESS: single  
224 (D) TOPOLOGY: linear  
225  
226 (ii) MOLECULE TYPE: peptide  
227  
228 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:  
229  
230 Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile  
231 1 5 10 15  
232  
233 Leu Lys  
234  
235  
236 (2) INFORMATION FOR SEQ ID NO:14:  
237  
238 (1) SEQUENCE CHARACTERISTICS:  
239 (A) LENGTH: 17 base pairs  
240 (B) TYPE: nucleic acid  
241 (C) STRANDEDNESS: single  
242 (D) TOPOLOGY: linear  
243  
244 (ii) MOLECULE TYPE: DNA (genomic)  
245  
246 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:  
247  
248 GCAGARGARA CNATHAA 17  
249  
250 (2) INFORMATION FOR SEQ ID NO:15:  
251  
252 (1) SEQUENCE CHARACTERISTICS:  
253 (A) LENGTH: 5 amino acids  
254 (B) TYPE: amino acid  
255 (C) STRANDEDNESS: single  
256 (D) TOPOLOGY: linear  
257  
258 (ii) MOLECULE TYPE: peptide

PAGE: 4

RAW SEQUENCE LISTING  
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153 Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
154 1 5 10 15
155
156 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
157 20 25 30
158
159 His Arg Gln Glu Ser Gly Phe Arg
160 35 40
161
162 (2) INFORMATION FOR SEQ ID NO:9:
163
164 (i) SEQUENCE CHARACTERISTICS:
165 (A) LENGTH: 21 base pairs
166 (B) TYPE: nucleic acid
167 (C) STRANDEDNESS: single
168 (D) TOPOLOGY: linear
169
170 (ii) MOLECULE TYPE: DNA (genomic)
171
172 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
173
174 CTGGAGTCGA CTGGCGGAC T 21
175
176 (2) INFORMATION FOR SEQ ID NO:10:
177
178 (i) SEQUENCE CHARACTERISTICS:
179 (A) LENGTH: 60 base pairs
180 (B) TYPE: nucleic acid
181 (C) STRANDEDNESS: single
182 (D) TOPOLOGY: linear
183
184 (ii) MOLECULE TYPE: DNA (genomic)
185
186 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
187
188 CCGCGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC 60
189
190 (2) INFORMATION FOR SEQ ID NO:11:
191
192 (i) SEQUENCE CHARACTERISTICS:
193 (A) LENGTH: 34 base pairs
194 (B) TYPE: nucleic acid
195 (C) STRANDEDNESS: single
196 (D) TOPOLOGY: linear
197
198 (ii) MOLECULE TYPE: DNA (genomic)
199
200 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
201
202 CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC 34
203
204 (2) INFORMATION FOR SEQ ID NO:12:
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100  
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102 (A) LENGTH: 33 base pairs  
103 (B) TYPE: nucleic acid  
104 (C) STRANDEDNESS: single  
105 (D) TOPOLOGY: linear  
106  
107 (ii) MOLECULE TYPE: DNA (genomic)  
108  
109 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  
110  
111 CCATCGATGG ATCTACCTG AAGCCGCTTT CTT 33  
112  
113 (2) INFORMATION FOR SEQ ID NO:6:  
114  
115 (1) SEQUENCE CHARACTERISTICS:  
116 (A) LENGTH: 17 base pairs  
117 (B) TYPE: nucleic acid  
118 (C) STRANDEDNESS: single  
119 (D) TOPOLOGY: linear  
120  
121 (ii) MOLECULE TYPE: DNA (genomic)  
122  
123 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:  
124  
125 ATTTAGGTGA CACTATA 17  
126  
127 (2) INFORMATION FOR SEQ ID NO:7:  
128  
129 (1) SEQUENCE CHARACTERISTICS:  
130 (A) LENGTH: 34 base pairs  
131 (B) TYPE: nucleic acid  
132 (C) STRANDEDNESS: single  
133 (D) TOPOLOGY: linear  
134  
135 (ii) MOLECULE TYPE: DNA (genomic)  
136  
137 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:  
138  
139 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34  
140  
141 (2) INFORMATION FOR SEQ ID NO:8:  
142  
143 (1) SEQUENCE CHARACTERISTICS:  
144 (A) LENGTH: 40 amino acids  
145 (B) TYPE: amino acid  
146 (C) STRANDEDNESS: single  
147 (D) TOPOLOGY: linear  
148  
149 (ii) MOLECULE TYPE: protein  
150  
151 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
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RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/585,895DATE: 04/11/96  
TIME: 14:24:20

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47 (D) TOPOLOGY: linear  
48  
49 (ii) MOLECULE TYPE: DNA (genomic)  
50  
51 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
52  
53 TGTCTCTGCT GTCCTTGTCT 20  
54  
55 (2) INFORMATION FOR SEQ ID NO:2:  
56  
57 (i) SEQUENCE CHARACTERISTICS:  
58 (A) LENGTH: 70 base pairs  
59 (B) TYPE: nucleic acid  
60 (C) STRANDEDNESS: single  
61 (D) TOPOLOGY: linear  
62  
63 (ii) MOLECULE TYPE: DNA (genomic)  
64  
65 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
66  
67 ACATGCATGC CACCATGCAG CGGGGGCGCG CGTGTGCCT GCGACTGTGG CTCTGCCTGG 60  
68  
69 GACTCCTGGA 70  
70  
71 (2) INFORMATION FOR SEQ ID NO:3:  
72  
73 (i) SEQUENCE CHARACTERISTICS:  
74 (A) LENGTH: 24 base pairs  
75 (B) TYPE: nucleic acid  
76 (C) STRANDEDNESS: single  
77 (D) TOPOLOGY: linear  
78  
79 (ii) MOLECULE TYPE: DNA (genomic)  
80  
81 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
82  
83 ACATGCATGC CCCGCCGTC ATCC 24  
84  
85 (2) INFORMATION FOR SEQ ID NO:4:  
86  
87 (i) SEQUENCE CHARACTERISTICS:  
88 (A) LENGTH: 22 base pairs  
89 (B) TYPE: nucleic acid  
90 (C) STRANDEDNESS: single  
91 (D) TOPOLOGY: linear  
92  
93 (ii) MOLECULE TYPE: DNA (genomic)  
94  
95 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
96  
97 CGGAATTCCC CATGACCCCA AC 22  
98  
99 (2) INFORMATION FOR SEQ ID NO:5:

TEAM 7

PAGE: 1

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PATENT APPLICATION US/08/585,895

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TIME: 14:24:17

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This Raw Listing contains the General  
Information Section and up to the first 5 pages.

SEQUENCE LISTING

ENTERED

(1) General Information:

(i) APPLICANT: Alitalo, Kari  
Joukov, Vladimir

(ii) TITLE OF INVENTION: Receptor Ligand

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun  
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive  
(C) CITY: Chicago  
(D) STATE: Illinois  
(E) COUNTRY: United States of America  
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Gass, David A.  
(B) REGISTRATION NUMBER: 38,153  
(C) REFERENCE/DOCKET NUMBER: 28113/33072

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 312/474-6300  
(B) TELEFAX: 312/474-0448  
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single

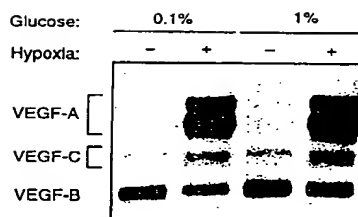


FIG. 18

FIG. 17

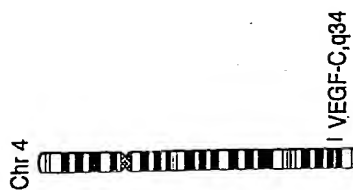


FIG. 15B

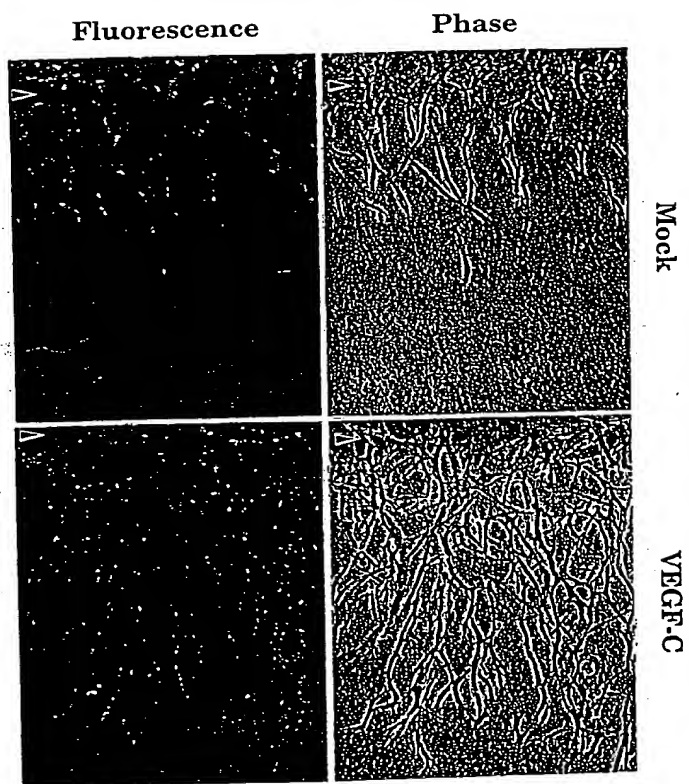




FIG. 15A

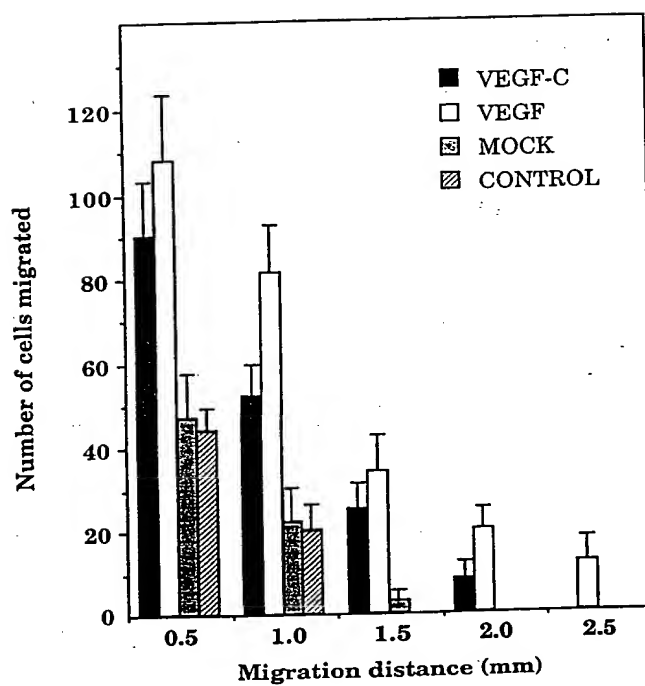
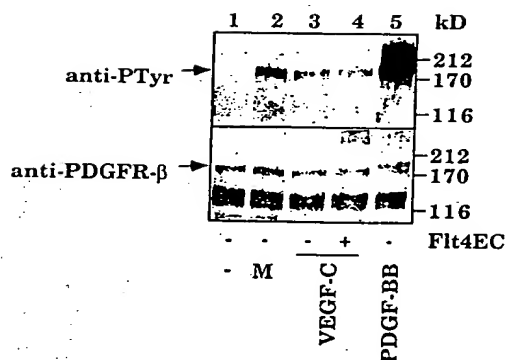


FIG. 14B



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FIG. 14A

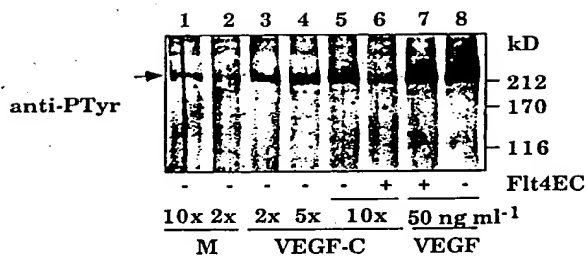
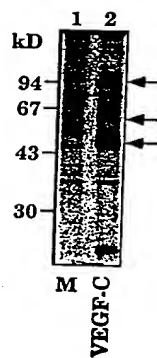


FIG. 13B



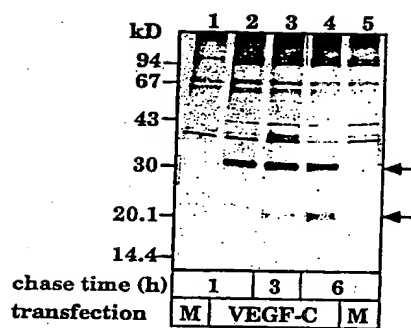


FIG. 13A

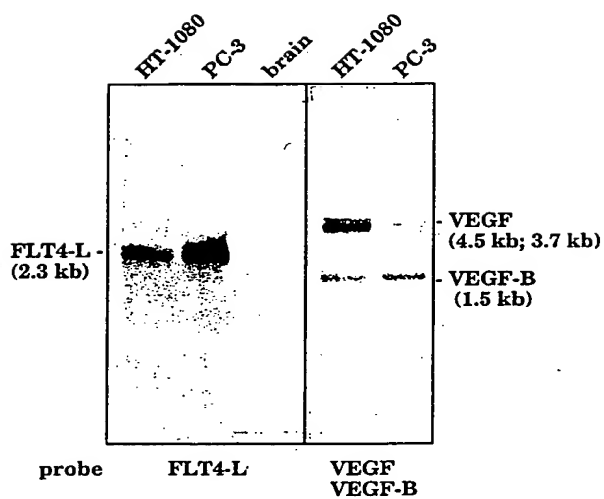


FIGURE 12

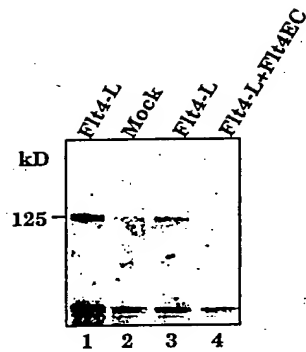


FIGURE 11

	251		300
PDGF-A	.....	.....	.....
PDGF-B	.....	.....	.....
PIGF-1	.....	.....	.....
PIGF-2	.....	.....	.....
VEGF121	.....	.....	.....
VEGF165	CGPCSEERRKH	LFVQDPQTCK	CSCKNVDSRC KARQLELNER TCRCDKPRR.
VEGF189	CGPCSEERRKH	LFVQDPQTCK	CSCKNVDSRC KARQLELNER TCRCDKPRR.
VEGF206	CGPCSEERRKH	LFVQDPQTCK	CSCKNVDSRC KARQLELNER TCRCDKPRR.
Flt4-L	FHDICGPNKE	LDEETCQCVC	RAGLAPASCG PHKELDRNSC QCVCKNGLFP
	301		350
PDGF-A	.....	.....	.....
PDGF-B	.....	.....	.....
PIGF-1	.....	.....	.....
PIGF-2	.....	.....	.....
VEGF121	.....	.....	.....
VEGF165	.....	.....	.....
VEGF189	.....	.....	.....
VEGF206	.....	.....	.....
Flt4-L	SQCGANREFD	ENTCQCCKR	TCPRNQPLNP GKACECTES PQKCLLKGNK
	351		394
PDGF-A	.....	.....	.....
PDGF-B	.....	.....	.....
PIGF-1	.....	.....	.....
PIGF-2	.....	.....	.....
VEGF121	.....	.....	.....
VEGF165	.....	.....	.....
VEGF189	.....	.....	.....
VEGF206	.....	.....	.....
Flt4-L	FHHQTCSCYR	RPCTNRQKAC	EPGFYSYSEEV CRCVPSYWKR PQMS

FIG. 10 B



50

1

PDGF-A .MRTWACILL LGGCYLANAL AEEAEIPREL IERLARSQIN SIRDLOKLEE  
 PDGF-B .MNRGWA.LFL SLGGCYLRVS AEGDPIPEEL YEMLSDBSIR SFDDLOKLEH  
 P1GF-1 .....MPVM RLFFC..FLQ LLAGLAL...  
 P1GF-2 .....M NPLLS..WVH WSLALLLYLH  
 VEGF121 .....M NPLLS..WVH WSLALLLYLH  
 VEGF165 .....M NPLLS..WVH WSLALLLYLH  
 VEGF189 .....M NPLLS..WVH WSLALLLYLH  
 VEGF206 .....M NPLLS..WVH WSLALLLYLH  
 Flt4-L .....M TVLYPEYMKH YKCQLRKGGM

51

PDGF-A IDSUGAEDAL ETSIRAHGSH AINHVPKRR VPIRRKRST...EEAIP  
 PDGF-B GDP.GEEDGA ELDLNMTIRSH SCGELES...LARGRRSLG SLTIAEPAM  
 P1GF-1 PAVPPQQW...ALSAG NGSEVEVVP PQE.VWGR...  
 P1GF-2 PAVPPQQW...ALSAG NGSEVEVVP PQE.VWGR...  
 VEGF121 HAKNSQAA...PMAEG GGQNHHEVVK FMD.VYOR...  
 VEGF165 HAKNSQAA...PMAEG GGQNHHEVVK FMD.VYOR...  
 VEGF189 HAKNSQAA...PMAEG GGQNHHEVVK FMD.VYOR...  
 VEGF206 HAKNSQAA...PMAEG GGQNHHEVVK FMD.VYOR...  
 Flt4-L QHNREQANLN SRTEETIKFA AARNYTEILK SIDNEWK...

100

101

PDGF-A AVCKTRTVIY EIPRSQVDPT SANFLIWPPC VEVKRCGCCC NTSSVVKQPS  
 PDGF-B AECKTRTEVF EISRRLLDRT NANFLVWPPC VEVQRCGCCC NNRNVQCRPT  
 P1GF-1 SYCRALERLV DVVSEYPS...EVERHFSFSC VSLLRCTGCC GDENLHCVPV  
 P1GF-2 SYCRALERLV DVVSEYPS...EVERHFSFSC VSLLRCTGCC GDENLHCVPV  
 VEGF121 SYCHPIETLV DIFOEYPD...EIEYIFKPS VPLMRGCGCC NDEGLECVPT  
 VEGF165 SYCHPIETLV DIFOEYPD...EIEYIFKPS VPLMRGCGCC NDEGLECVPT  
 VEGF189 SYCHPIETLV DIFOEYPD...EIEYIFKPS VPLMRGCGCC NDEGLECVPT  
 VEGF206 SYCHPIETLV DIFOEYPD...EIEYIFKPS VPLMRGCGCC NDEGLECVPT  
 Flt4-L TQCHPREVCI DVGKEFGV...ATNITFKPPC VSVYRCGCCC NSEGLQCMYT

151

PDGF-A RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEACAT...SN  
 PDGF-B QVQLRPVQVR KIEIVRKXPI FKATVTLED HLACKETVA AARPVTRSPG  
 P1GF-1 ETANVTMQLL KIRSC...DRP .SYVELTFSQ HVRCECRPLR EKMKPER...  
 P1GF-2 ETANVTMQLL KIRSC...DRP .SYVELTFSQ HVRCECRPLR EKMKPER...  
 VEGF121 EESNITMQIM RIKPH...CQG .HIGEMSFLO HNKCECRPKK DRARQEKCD...  
 VEGF165 EESNITMQIM RIKPH...CQG .HIGEMSFLO HNKCECRPKK DRARQEKCD...  
 VEGF189 EESNITMQIM RIKPH...CQG .HIGEMSFLO HNKCECRPKK DRARQEKKS...  
 VEGF206 EESNITMQIM RIKPH...CQG .HIGEMSFLO HNKCECRPKK DRARQEKKS...  
 Flt4-L STSYLSKTLF EITVPLSQGP .KPVTIISFAN HTSCRCHSKL DVYRQVHSII

200

201

PDGF-A LNPDRHEET DVR.....  
 PDGF-B GSQEQRAKTP QTRVTIRTVR VRRPPKGRH KFKNTHDKTA LKETLGA...  
 P1GF-1 .....CGDAVPR R.....  
 P1GF-2 .....PKGRGK RRREKQRPD CHLCGDAVPR R.....  
 VEGF121 .....KPRR.....  
 VEGF165 .....VRGKGR GQKRKRKKS YKSWSV.....  
 VEGF189 .....VRGKGR GQKRKRKKS YKSWSVYVGA RCC.....L MPWSLPGPH  
 VEGF206 .....VRGKGR GQKRKRKKS YKSWSVYVGA RCC.....L MPWSLPGPH  
 Flt4-L RRSLPATLPQ CQAANKTCPT NYHWRNHICR CLAQEDPHFS SDAGDDSTDG

FIG. 10A

	251				300
PDGF-A	.....	.....	.....	.....	.....
PDGF-B	.....	.....	.....	.....	.....
P1GF-1	.....	.....	.....	.....	.....
P1GF-2	.....	.....	.....	.....	.....
VEGF121	.....	.....	.....	.....	.....
VEGF165	CGPCSERRKH	LFVQDPQTCK	CSCKNYDSRC	KARQLELNER	TCRCDKPRR.
VEGF189	CGPCSERRKH	LFVQDPQTCK	CSCKNYDSRC	KARQLELNER	TCRCDKPRR.
VEGF206	CGPCSERRKH	LFVQDPQTCK	CSCKNYDSRC	KARQLELNER	TCRCDKPRR.
Flt4-L	FHDICGPNKE	LDEETCQCVC	RAGLRPASCG	PHKELDRNSC	QCVCNKLF
	301				350
PDGF-A	.....	.....	.....	.....	.....
PDGF-B	.....	.....	.....	.....	.....
P1GF-1	.....	.....	.....	.....	.....
P1GF-2	.....	.....	.....	.....	.....
VEGF121	.....	.....	.....	.....	.....
VEGF165	.....	.....	.....	.....	.....
VEGF189	.....	.....	.....	.....	.....
VEGF206	.....	.....	.....	.....	.....
Flt4-L	SQCGANREFD	ENTCQCVCCKR	TCPRNQPLNP	GKCACTES	PQRCLLKGGK
	351				394
PDGF-A	.....	.....	.....	.....	.....
PDGF-B	.....	.....	.....	.....	.....
P1GF-1	.....	.....	.....	.....	.....
P1GF-2	.....	.....	.....	.....	.....
VEGF121	.....	.....	.....	.....	.....
VEGF165	.....	.....	.....	.....	.....
VEGF189	.....	.....	.....	.....	.....
VEGF206	.....	.....	.....	.....	.....
Flt4-L	FHHQTCSCYR	RPCTNRQKAC	EPGFSYSEEV	CRCVPSYWKR	PQMS

FIG. 10

1  
 PDGF-A .MRTWACLLL LCCGYLAHAL AEEAEIPREL IERLARSQIH SIRDLORLLE 50  
 PDGF-B MNRCWA.LFL SLCCYLRLVS AEGDPIPEEL YEMLSDHSIR SFDDLQRLH  
 PIGF-1 .....MPVM RLFPCC..FLQ LLAGLAL...  
 PIGF-2 .....MPVM RLFPCC..FLQ LLAGLAL...  
 VEGF121 .....M NFLLS..WVH WSLALLLYLH  
 VEGF165 .....M NFLLS..WVH WSLALLLYLH  
 VEGF189 .....M NFLLS..WVH WSLALLLYLH  
 VEGF206 .....M NFLLS..WVH WSLALLLYLH  
 Flt4-L .....M TVLYPEYWKM YXCQLRKGW

51  
 PDGF-A IDSUGAEDAL ETSLRANGSH AINHVPKPRP VPIRRKRSI. ....EEAIP 100  
 PDGF-B GDP.GEEDGA ELDLNMTRSH SGGELSES... .LARGRRSLG SLTIAEPAMI  
 PIGF-1 PAVPPQQW... .ALSAG NGSSSEVVP FQE.VMGR...  
 PIGF-2 PAVPPQQW... .ALSAG NGSSSEVVP FQE.VMGR...  
 VEGF121 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...  
 VEGF165 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...  
 VEGF189 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...  
 VEGF206 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...  
 Flt4-L QHNREQANLN SRTEETIKFA AAHYNTEILK SIDNEWRR...

101  
 PDGF-A AVCKTRTVIY EIPRSQVDPT SANFLIWPPC VEVKRCTGCC NTSSVKCQPS 150  
 PDGF-B AECKTRTEVF EISRLIDRT NANFLWPPC VEVRQCSGCC NNRNVQCRPT  
 PIGF-1 SYCRALERLV DUVSEYPS... EVEHMFSPSC VSLLRCTGCC GDENLHCVPV  
 PIGF-2 SYCRALERLV DUVSEYPS... EVEHMFSPSC VSLLRCTGCC GDENLHCVPV  
 VEGF121 SYCHFIETLV DIFQEYPD... EIEYIFKPS VPLMRCGGCC NDEGLECVPT  
 VEGF165 SYCHFIETLV DIFQEYPD... EIEYIFKPS VPLMRCGGCC NDEGLECVPT  
 VEGF189 SYCHFIETLV DIFQEYPD... EIEYIFKPS VPLMRCGGCC NDEGLECVPT  
 VEGF206 SYCHFIETLV DIFQEYPD... EIEYIFKPS VPLMRCGGCC NDEGLECVPT  
 Flt4-L TQCMFREVCI DVGKEFGV... ATNTFFKPPC VSVYRCGGCC NSEGLQCMNT

151  
 PDGF-A RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEACAT... .SN 200  
 PDGF-B QVQLRPVQVR KIEIVRKKPI FKATVTLED HLAACKCETVA AARPVTRSPG  
 PIGF-1 ETANVTMQLL KIRSG...DRP .SYVELTFSQ HVRCECRPLR EKMKPER...  
 PIGF-2 ETANVTMQLL KIRSG...DRP .SYVELTFSQ HVRCECRPLR EKMKPER...  
 VEGF121 EESNITMQIM RIKPH...GGQ .HIGEMSFLQ HNKCECRPKK DRARQEKCD.  
 VEGF165 EESNITMQIM RIKPH...GGQ .HIGEMSFLQ HNKCECRPKK DRARQEN...  
 VEGF189 EESNITMQIM RIKPH...GGQ .HIGEMSFLQ HNKCECRPKK DRARQEKKS.  
 VEGF206 EESNITMQIM RIKPH...GGQ .HIGEMSFLQ HNKCECRPKK DRARQEKKS.  
 Flt4-L STSYLSKTLF EITVPLSQGP .KPVITISFAN HTSCRCHMSKL DVYRQVHSII

201  
 PDGF-A LNPDHREET DVR..... 250  
 PDGF-B GSQEQRATP QTRVTIRTVR VRRPPKGKHR KFKHTHDKTA LKETLGA...  
 PIGF-1 .....CGDAVPR R.....  
 PIGF-2 .....PKGRGK RRREKQRPD CHLCGDAVPR R.....  
 VEGF121 .....KPRR.....  
 VEGF165 .....P  
 VEGF189 .....VRGKCK GQKRKRKSR YKSWSV.....P  
 VEGF206 .....VRGKCK GQKRKRKSR YKSWSVYGA RCC.....L MPWSLPGPH  
 Flt4-L RRLPATLPQ CQAANKTCPT NYMNNNHICR CLAQEDFMS SDAGDDSDTG

FIG. 10

MetThrValLeuTyrProGluTyr  
GACCAGTTACGGTCTGTGTCCACTCTGACTCAACTCATCACTCTACTCTACCCAGAATAT  
10 30 50  
TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla  
TGGAAATGTACAGTGTTCAGCTAAGAAAGGAGGCTGCCAACATAACAGAGAACAGGCC  
70 90 110  
AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaHisTyrAsnThrGlu  
AACCTCACTCAAGCAGAGAGACTATAAAATTTGCTCCAGCACATTATAATACAGAG  
130 150 170  
IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys  
ATCTTGAAAGTATTGATATGACTGGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT  
190 210 230  
IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal  
ATAGATCTGCGCAAGGAGTTTGGAGTCGGCAGAACACCTTCTTTAAACCTCCATCTGTG  
250 270 290  
SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer  
TCCGTCTACAGATCTGCGGGTTGCTGCAATAGTACGGGGTGCAGTGCATCAACACCAGC  
310 330 350  
ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys  
ACGAGCTACCTCAGCAAGAGCTTATTGAAATTACAGTCCCTCTCTCAAGGCCCAAA  
370 390 410  
ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal  
CCAGTAACATCAGTTTTGCCAATCACACTTCTGCGGATGCATCTCTAACTGGATGTT  
430 450 470  
TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln  
TACAGACAAGTTCATTCATTATTAGAGCTTCCCTGCCAGCAACACTACCACAGTGTGAG  
490 510 530  
AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu  
CGAGCGAACAGAGCTGCCCCACCAATTACATGTGGAATATCACATCTCAGATGCTCTG  
550 570 590  
AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis  
CCTCAGCAGATTTTATGTTTTCTCTCGATGCTGGAGATGACTCAACAGATGGATTCCAT  
610 630 650  
AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla  
CACATCTGTGACCAACAGAGCTGTGATGAAGAGACCTGTCACTGTCTCTCGAGAGCG  
670 690 710  
GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys  
GGGCTTGGGCTGCCAGCTGTGACCCCCACAAAGAACTAGACAGAACTCATGCCAGTGT  
730 750 770  
ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn  
CTCTGTAAACAAACTCTTCCCCAGCCAATGTGGGGCAACCGAGAATTTCATGAAAC  
790 810 830  
ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys  
ACATGCCAGTGTGTATGTAAAGAACCTGCCCCAGAAATCAACCCCTAAATCTCGAAAA  
850 870 890  
CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis  
TGTGCTGTGAAATGTACAGAAAGTCCACAGAAATGCTTGTAAAGGAAAGAGATTCAC  
910 930 950  
HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro  
CACCACAACTTCAGCTGTTACAGACGGCCATGTACGAACCGCCAGAGGCTGTGAGCCA  
970 990 1010  
GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln  
CGATTTTCATATAGTCAACAAGTGTCTGCTTCTGCTCCCTTCATATGGAAAAAGCCACAA  
1030 1050 1070  
MetSerEnd  
ATGAGCTAAGATTGTACTGTTTTCCAGTTCACTGATTTTCTATTATGAAAAACTGTGTTG  
1090 1110 1130

FIG. 9B

FIG. 9A

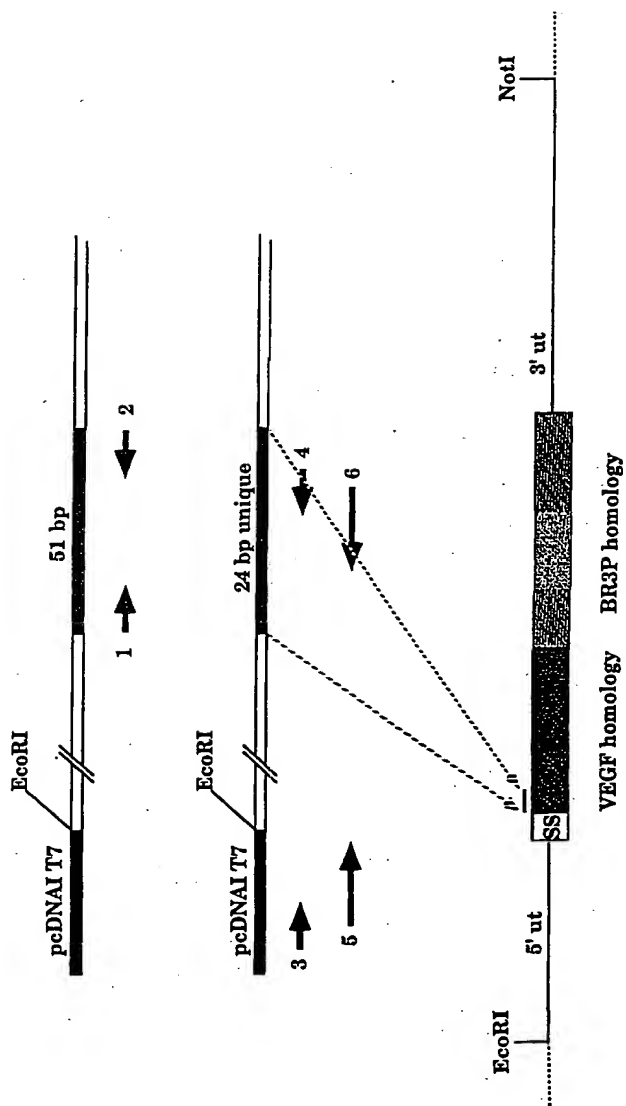
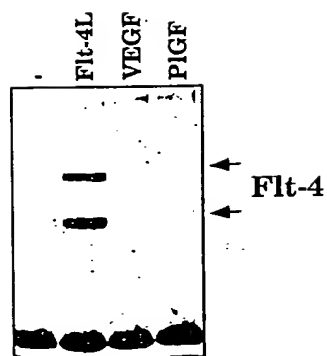


FIGURE 8



08 585895

FIGURE 7

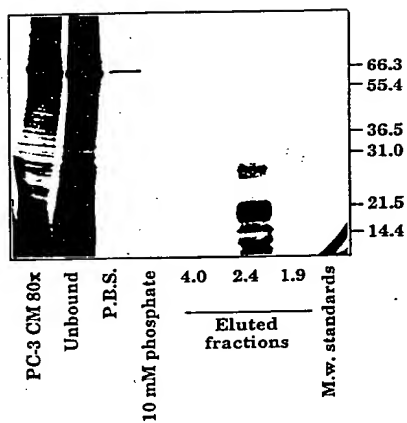


FIGURE 7

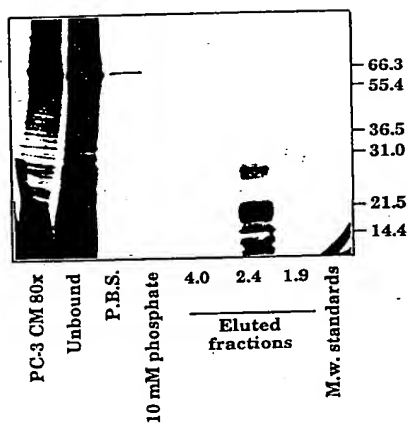
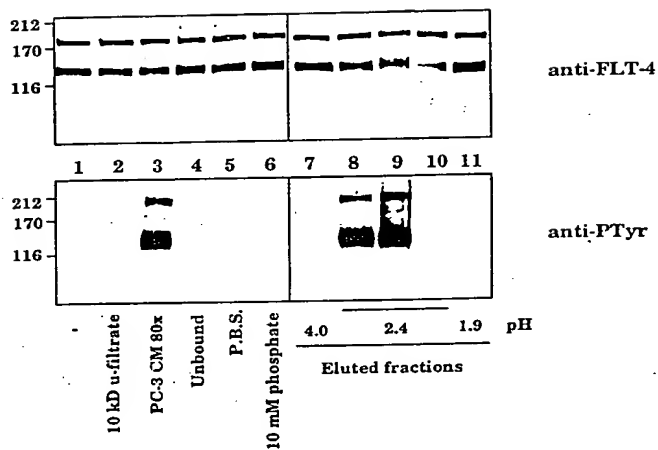




FIGURE 6



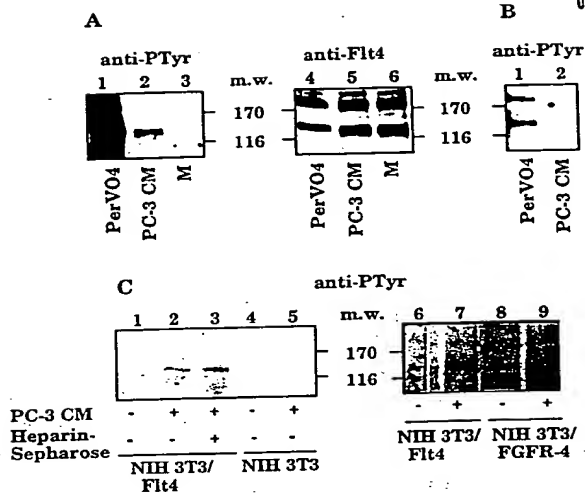


FIGURE 5

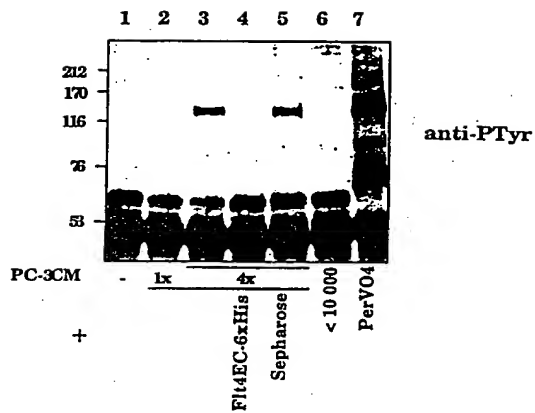
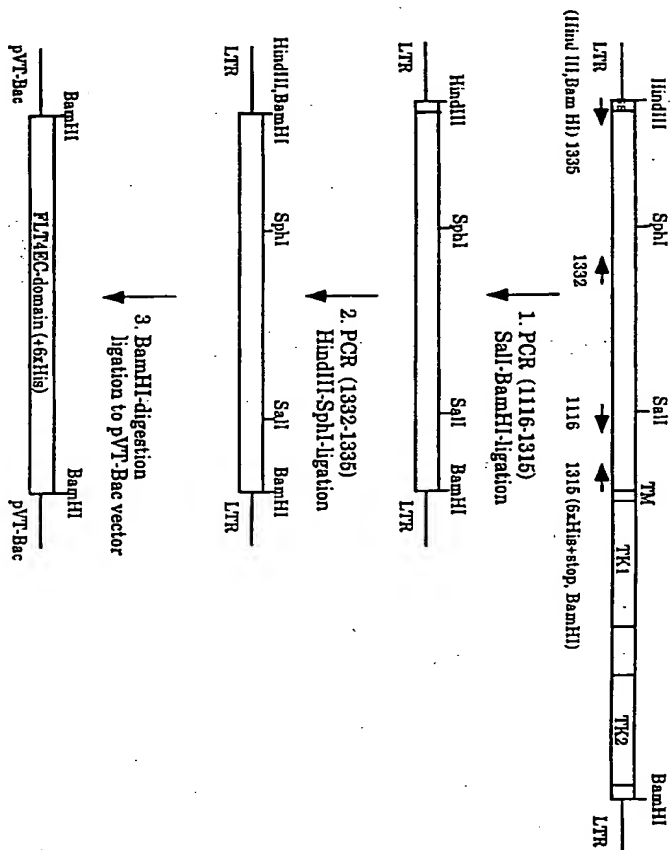


FIGURE 4

Figure 3



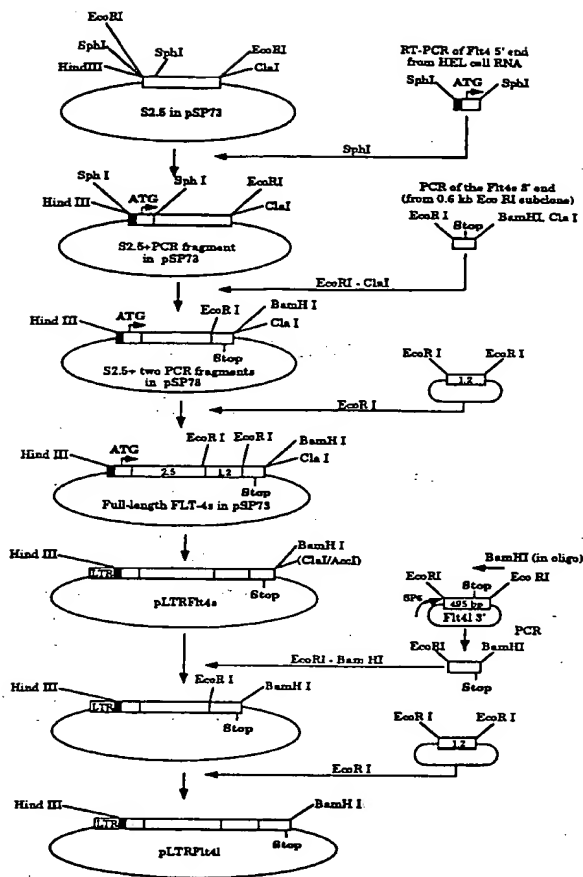


FIGURE 2

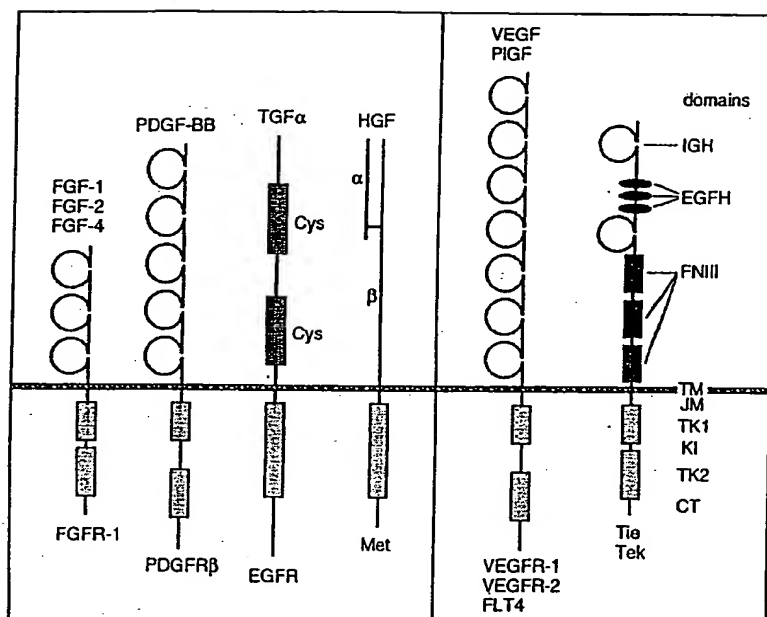


FIGURE 1

-57-

12. The fragment according to claim 8 comprising approximately amino acids 1-180 shown in SEQ ID NO: 33.

13. A purified and isolated nucleic acid encoding the fragment of claim 12.

14. An antibody which is specifically reactive with the Flt4 ligand.

15. An antibody of claim 14 which is a monoclonal antibody.

16. A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

Sub 331

add B6

add  
C10

add  
D12

61  
-58-  
CLAIMS

- Sub B1
1. A purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase.
  2. A purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.
  3. A purified and isolated nucleic acid encoding the peptide according to claim 2.
  4. The nucleic acid according to claim 3 having the sequence shown in SEQ ID NO: 32.
  5. A vector comprising the nucleic acid according to claim 4.
  6. The vector according to claim 5, wherein said vector is plasmid pFLT4-L, deposited as ATCC accession No. 97231.
  7. A host cell comprising the vector according to claim 6.
  8. A fragment of the purified and isolated polypeptide according to claim 2, said fragment being capable of specifically binding to an Flt4 receptor tyrosine kinase.
  9. The fragment according to claim 8 having an apparent molecular weight of 23 kD under reducing conditions.
  10. The fragment according to claim 8 comprising approximately amino acids 1-120 of SEQ ID NO: 33.
  11. A purified and isolated nucleic acid encoding the fragment of claim 10.
- Sub B3
- Sub B4/CS
- Sub C6
- Sub B5



Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu  
 115 120 125  
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr  
 130 135 140  
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp  
 145 150 155 160  
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His  
 165 170 175  
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys  
 180 185 190  
 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu  
 195 200 205  
 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro  
 210 215 220  
 Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys  
 225 230 235 240  
 Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys  
 245 250 255  
 Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly  
 260 265 270  
 Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr  
 275 280 285  
 Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val  
 290 295 300  
 Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser  
 305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGAGTGATTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCAACCCCATCT

22

CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC	774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys	
200 205 210	
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC	822
Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn	
215 220 225 230	
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC	870
Arg Glu Phe Asp Gln Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys	
235 240 245	
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA	918
Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr	
250 255 260	
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA	966
Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Phe His His Gln	
265 270 275	
ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT	1014
Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys	
280 285 290	
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA	1062
Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser	
295 300 305 310	
TAT TGG AAA AGA CCA CAA ATG AGC TAA GATTGTACTG TTTTCCAGTT	1109
Tyr Trp Lys Arg Pro Gln Met Ser	
315	
CATCGATTIT CTATTATGGA AAACGTGTIT G	1140

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu	
-32 -30 -25 -20	
Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser	
-15 -10 -5	
Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu	
1 5 10 15	
Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro	
20 25 30	
Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn	
35 40 45	
Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys	
50 55 60	
Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu	
65 70 75 80	
Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys	
85 90 95	
Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser	
100 105 110	

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 37..1089

(ix) FEATURE:

(A) NAME/KEY: mat\_peptide

(B) LOCATION: 133..1089

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC	GGTCTGTGTC	CAGTGTAGAT	GAACCTC	ATG	ACT	GTA	CTC	TAC	CCA	54							
				Met	Thr	Val	Leu	Tyr	Pro								
				-32		-30											
GAA	TAT	TGG	AAA	ATG	TAC	AAG	TGT	CAG	CTA	AGG	AAA	GGA	GGC	TGG	CAA	102	
Glu	Tyr	Trp	Lys	Met	Tyr	Lys	Cys	Gln	Leu	Arg	Lys	Gly	Gly	Trp	Gln		
	-25			-20								-15					
CAT	AAC	AGA	GAA	CAG	GCC	AAC	CTC	AAC	TCA	AGG	ACA	GAA	GAG	ACT	ATA	150	
His	Asn	Arg	Glu	Gln	Ala	Asn	Leu	Asn	Ser	Arg	Thr	Glu	Glu	Thr	Ile		
	-10			-5						1				5			
AAA	TTT	GCT	GCA	GCA	CAT	TAT	TAT	AAT	ACA	GAG	ATC	TTG	AAA	AGT	ATT	GAT	198
Lys	Phe	Ala	Ala	Ala	His	Tyr	Asn	Thr	Glu	Ile	Leu	Lys	Ser	Ile	Asp		
	10							15						20			
AAT	GAG	TGG	AGA	AAG	ACT	CAA	TGC	ATG	CCA	CGG	GAG	GTG	TGT	ATA	GAT	246	
Asn	Glu	Trp	Arg	Lys	Thr	Gln	Cys	Met	Pro	Arg	Glu	Val	Cys	Ile	Asp		
	25						30					35					
GTG	GGG	AAG	GAG	TTT	GGA	GTC	CGC	ACA	AAC	ACC	TTC	TTT	AAA	CCT	CCA	294	
Val	Gly	Lys	Glu	Phe	Gly	Val	Ala	Thr	Asn	Thr	Phe	Phe	Lys	Pro	Pro		
	40				45						50						
TGT	GTG	TCC	GTC	TAC	AGA	TGT	GGG	GGT	TGC	TGC	AAT	AGT	GAG	GGG	CTG	342	
Cys	Val	Ser	Val	Tyr	Arg	Cys	Gly	Gly	Cys	Asn	Ser	Glu	Gly	Leu			
	55			60					65					70			
CAG	TGC	ATG	AAC	ACC	AGC	ACG	AGC	TAC	CTC	AGC	AAG	ACG	TTA	TIT	GAA	390	
Gln	Cys	Met	Asn	Thr	Ser	Thr	Ser	Tyr	Leu	Ser	Lys	Thr	Leu	Phe	Glu		
				75					80					85			
ATT	ACA	GTG	CCT	CTC	TCT	CAA	GGC	CCC	AAA	CCA	GTA	ACA	ATC	AGT	TTT	438	
Ile	Thr	Val	Pro	Leu	Ser	Gln	Gly	Pro	Lys	Pro	Val	Thr	Ile	Ser	Phe		
	90							95					100				
GCC	AAT	CAC	ACT	TCC	TGC	CGA	TGC	ATG	TCT	AAA	CTG	GAT	GTT	TAC	AGA	486	
Ala	Asn	His	Thr	Ser	Cys	Arg	Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg		
	105						110					115					
CAA	GTT	CAT	TCC	ATT	ATT	AGA	CGT	TCC	CTG	CCA	GCA	ACA	CTA	CCA	CAG	534	
Gln	Val	His	Ser	Ile	Ile	Arg	Arg	Ser	Leu	Pro	Ala	Thr	Leu	Pro	Gln		
	120					125					130						
TGT	CAG	GCA	CGC	AAC	AAG	ACC	TGC	CCC	ACC	AAT	TAC	ATG	TGG	AAT	AAT	582	
Cys	Gln	Ala	Ala	Asn	Lys	Thr	Cys	Pro	Thr	Asn	Tyr	Met	Trp	Asn	Asn		
	135			140					145					150			
CAC	ATC	TGC	AGA	TGC	CTG	GCT	CAG	GAA	GAT	TTT	ATG	TTT	TCC	TGG	GAT	630	
His	Ile	Cys	Arg	Cys	Leu	Ala	Gln	Glu	Asp	Phe	Met	Phe	Ser	Ser	Asp		
				155					160					165			
GCT	GGA	GAT	GAC	TCA	ACA	GAT	GGA	TTC	CAT	GAC	ATC	TGT	GGA	CCA	AAC	678	
Ala	Gly	Asp	Ser	Thr	Asp	Gly	Phe	His	Asp	Ile	Cys	Gly	Pro	Asn			
	170					175						180					
AAG	GAG	CTG	GAT	GAA	GAG	ACC	TGT	CAG	TGT	GTC	TGC	AGA	GCG	GGG	CTT	726	
Lys	Glu	Leu	Asp	Glu	Glu	Thr	Cys	Gln	Cys	Val	Cys	Arg	Ala	Gly	Leu		
	185					190						195					

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA (genomic)  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATT AGGTGACAC

19

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTGCT GCAGC

25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTGA

20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACTATAGG GAGACCCAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1140 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:  
GTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn  
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 219 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT

60

GTGGTGAAT TCGAAGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG

120

TGTGAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG

180

ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC

219

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC

18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid

Thr Glu Ile Leu Lys  
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCNGTGTGT AGTGTCGTG

19

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala His Tyr Asn Thr Glu Ile  
1 5 10 15  
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CNATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Glu Thr Ile Lys  
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCATTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
1           5           10           15
Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
20           25           30
His Arg Gln Glu Ser Gly Phe Arg
35           40

```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTCCTGTG ATGTGACCA

20



(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCCGCCGTC ATCC 24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCT CATGACCCCA AC 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTTAGGTGA CACTATA 17

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 amino acids



- 40 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari  
Joukov, Vladimir
- (ii) TITLE OF INVENTION: Receptor Ligand
- (iii) NUMBER OF SEQUENCES: 25
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun  
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive  
(C) CITY: Chicago  
(D) STATE: Illinois  
(E) COUNTRY: United States of America  
(F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Gass, David A.  
(B) REGISTRATION NUMBER: 38,153  
(C) REFERENCE/DOCKET NUMBER: 28113/33072
- (ix) TELECOMMUNICATION INFORMATION:  
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(B) TELEFAX: 312/474-0448  
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 70 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGGCGCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG  
GACTCCTGGA

60

70

(2) INFORMATION FOR SEQ ID NO:3:

isolated (as in the other examples), and 8 micrograms of the RNA was electrophoresed and blot-hybridized with a mixture of the VEGF, VEGF-B and VEGF-C probes (see Fig. 12). The results show that hypoxia strongly induces VEGF-A mRNA expression (compare lanes - and +), both in low and high glucose, but has no significant effect on the VEGF-B mRNA levels. The VEGF-C mRNA isolated from hypoxic cells runs slightly faster in gel electrophoresis and an extra band of faster mobility can be seen below the upper mRNA band. This observation suggests that hypoxia affects VEGF-C RNA processing. One explanation for this observation is that VEGF-C mRNA splicing is altered, affecting the VEGF-C open reading frame and resulting in an alternative VEGF-C protein being produced by hypoxic cells. Such alternative forms of VEGF-C and VEGF-C-encoding polynucleotides are contemplated as an aspect of the invention.

Deposit of Biological Materials: Plasmid FLT4-L has been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville MD <sup>20852</sup> 20952 (USA), pursuant to the provisions of the Budapest Treaty, and has been assigned a deposit date of 24 July 1995 and ATCC accession number 97231.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

In order to determine the chromosomal localization of the human VEGF-C gene, DNAs from human rodent somatic cell hybrids containing defined sets of human chromosomes were <sup>analysed</sup> by Southern blotting and hybridization with the VEGF-C cDNA probe. Among 24 DNA samples on the hybrid panel, representing different human chromosomes, human-specific signals were observed only in hybrids which contained human chromosome 4. The results were confirmed by PCR of somatic cell hybrid DNA using VEGF-C specific primers, where amplified bands were obtained only from DNAs containing human chromosome 4.

A genomic P1 plasmid for VEGF-C was isolated using specific primers and PCR and verified by Southern blotting and hybridization using a VEGF-C specific cDNA probe. The chromosomal localization of VEGF-C was further studied using metaphase FISH. Using the P1 probe for VEGF-C in FISH a specific hybridization to the 4q34 chromosomal band was detected in 40 out of 44 metaphases (Fig. 17). Double-fluorochrome hybridization using a cosmid probe specific for the aspartylglucosaminidase (AGA) gene showed that VEGF-C is located just proximal to the AGA gene previously mapped to the 4q34-35 chromosomal band.

Biotin labelled VEGF-C P1 and digoxigenin labeled AGA cosmid probes were hybridized simultaneously to metaphase chromosomes. This experiment demonstrated that the AGA gene is more telomerically located than the VEGF-C gene. The foregoing example demonstrates the utility of polynucleotides of the invention as chromosomal markers.

#### EXAMPLE 18

##### Effect of glucose concentration and hypoxia on VEGF, VEGF-B and VEGF-C mRNA levels in C6 glioblastoma cells

Confluent cultures of C6 cells (ATCC CCL 107) were grown on 10 cm diameter tissue culture plates containing 2.5 ml of DMEM and 5% fetal calf serum plus antibiotics. The cultures were exposed for 16 hours to normoxia in a normal cell culture incubator containing 5% CO<sub>2</sub> (Fig. 18: lanes marked -) or hypoxia (Fig. 18: lanes marked +) by closing the culture plates in an airtight glass chamber and burning a piece of wood inside until the flame was extinguished due to lack of oxygen. Polyadenylated RNA was

domain was used as a probe in Southern blotting and hybridization analysis of the somatic cell hybrid DNAs as instructed by the supplier (Bios Laboratories).

The cell lines for fluorescence *in situ* hybridization (FISH) were obtained from the American Type Culture Collection (Rockville, MD).

5 Purified DNA from P1 clones 7660 and 7661 (VEGF-C) (Genome Systems, Inc., St. Louis, MO) were confirmed positive by Southern blotting of Eco RI-digested DNA followed by hybridization with the VEGF-C cDNA. The P1 clones were then labelled by nick translation either with biotin-11-dUTP, biotin-14-ATP (Sigma Chemical Co., St. Louis, MO) or digoxigenin 11-dUTP  
10 (Boehringer Mannheim GmbH, Mannheim, Germany) according to standard protocols. PHA-stimulated peripheral blood lymphocyte cultures were treated with 5-bromodeoxyuridine (BrdU) at an early replicating phase to induce G-banding. See Takahashi *et al.*, *Human Genet.*, 86:14-16 (1995); Lemieux *et al.*, *Cytogenet. Cell Genet.*, 59:311-12 (1992). The FISH procedure was  
15 carried out in 50% formamide, 10% dextran sulphate in 2x SSC using well-known procedures. See, e.g., Rytkönnen *et al.*, *Cytogenet. Cell Genet.*, 68:61-63 (1995); Lichter *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:9664-68 (1988). Repetitive sequences were suppressed with 50-fold excess of Cot-1  
C DNA (BRL, Gaithersburg, MD) compared with the labeled probe. Specific  
20 hybridization signals were detected by incubating the hybridized slides in labelled antidigoxigenin antibodies, followed by counterstaining with 0.1mmol/L 4,6-diamino-2-phenylindole. Probe detection for two-color experiments was accomplished by incubating the slides in fluorescein isothiocyanate (FITC)-anti-digoxigenin antibodies (Sigma Chemical Co.) and  
25 Texas red-avidin (Vector Laboratories, Burlingame, CA) or rhodamine-anti-digoxigenin and FITC-avidin.

Multi-color digital image analysis was used for acquisition, display and quantification of hybridization signals of metaphase chromosomes. The system contains a PXL camera (Photometrics Inc., Tucson, AZ) attached  
30 to a PowerMac 7100/Av workstation. IPLab software controls the camera operation, image acquisition and Ludl Filter wheel. At least 50 nuclei were scored. Overlapping nuclei and clusters of cells were ignored. A slide containing normal lymphocyte metaphase spreads and interphase nuclei was included in each experiment to control for the efficiency and specificity of the  
35 hybridization.

example of typical phase contrast and fluorescent microscopic fields of cultures stimulated with medium from mock-transfected or VEGF-C transfected cells is shown in Fig. 15B. Daily addition of 1 ng of FGF2 into the wells resulted in the migration of approximately twice the number of cells when compared to the stimulation by CM from VEGF-transfected cells.

#### EXAMPLE 16

##### VEGF-C Is Expressed In Multiple Tissues

Northern blots containing 2 micrograms of isolated poly(A)<sup>+</sup> RNA from multiple human tissues (blot from Clontech) were probed with radioactively labelled insert of the 2.0 kb VEGF-C cDNA clone. Northern blotting and hybridization analysis showed that the 2.4 kb RNA and smaller amounts of a 2.0 kb mRNA are expressed in multiple human tissues, most prominently in the heart, placenta, muscle, ovary and small intestine (Fig. 16A). Very little VEGF-C RNA was seen in the brain, liver or thymus and peripheral blood leukocytes (pbl) appeared negative. A similar analysis of RNA from human fetal tissues (Fig. 16B) shows that VEGF-C is highly expressed in the kidney and lung and to a lesser degree in the liver, while essentially no expression is detected in the brain. Interestingly, VEGF expression correlates with VEGF-C expression in these tissues, whereas VEGF-B is <sup>highly</sup> expressed in all tissues <sup>analysed</sup>.

#### EXAMPLE 17

##### The VEGF-C Gene Localizes To Chromosome 4q34

A DNA panel of 24 interspecies somatic cell hybrids, which had retained one or two human chromosomes, was used for the chromosomal localization of the VEGF-C gene (Bios Laboratories, Inc., New Haven, CT). Primers were designed to amplify an about 250 bp fragment of the VEGF-C gene from somatic cell hybrid DNA. The primers and conditions for polymerase chain reaction (PCR) were 5'-TGAGTGATTTGTAGCTGCTGTG-3' (forward) [SEQ ID NO:34] and 5'-TATTGCAGCAACCCCCACATCT-3' (reverse) [SEQ ID NO:35] for VEGF-C (94°C, 60s/62°C, 45s/72°C, 60s). The PCR products were evaluated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining in ultraviolet light. [ $\alpha$ -<sup>32</sup>P]-dCTP-labelled cDNA inserts of a plasmid representing the complete VEGF-C coding

For the migration assays, the cells were allowed to attach inside a plastic ring (1 cm diameter) placed on top of the first collagen layer. After 30 min., the ring was removed and unattached cells were rinsed away. A second layer of collagen and a layer of growth medium (5% newborn calf serum (NCS)), solidified by 0.75% low melting point agar (FMC BioProducts, Rockland, ME), were added. A well (3 mm diameter) was punched through all the layers on both sides of the cell spot at a distance of 4 mm, and the sample or control media were pipetted daily into the wells. Photomicrographs of the cells migrating out from the spot edge were taken after six days through an Olympus CK 2 inverted microscope equipped with phase-contrast optics. The migrating cells were counted after nuclear staining with the fluorescent dye bisbenzimidazole (1 mg/ml, Hoechst 33258, Sigma).

Fig. 15A depicts a comparison of the number of cells migrating at different distances from the original area of attachment towards wells containing media conditioned by the non-transfected (control) or transfected (mock; VEGF-C; VEGF) cells, 6 days after addition of the media. The number of cells migrating out from the original ring of attachment was counted in five adjacent 0.5 mm x 0.5 mm squares using a microscope ocular lens grid and 10x magnification. Cells migrating further than 0.5 mm were counted in a similar way by moving the grid in 0.5 mm steps. The experiments were carried out twice with similar results, and medium values from the one of the experiments are presented with standard error bars. The photographs in Fig. 15B depict phase-contrast microscopy and fluorescent microscopy of the nuclear staining of BCE cells migrating towards the wells containing media conditioned by the mock-transfected cells or by VEGF-C - transfected cells. The areas shown is approximately 1mm x 1.5mm, and arrows indicate the borders of the original ring of attachment.

After 6 days of treatment, the cultures were stained and cells at different distances outside of the original ring of attachment were counted using fluorescent nuclear staining and detection with a fluorescence microscope equipped with a grid. A comparison of the numbers of migrating cells in successive 0.5 mm x 0.5 mm areas is shown in Fig 15A. As can be seen from the columns, VEGF-C-containing CM stimulated cell migration more than medium conditioned by the non-transfected or mock-transfected cells but less than medium from cells transfected with a VEGF expression vector. An

nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH<sub>2</sub>-terminal sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in carboxy-terminal truncations of its protein product. The activities of such truncated forms are assayed by, for example, studying Flt4 autophosphorylation induced by the truncated proteins when applied to cultures of cells, such as NIH3T3 cells expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin *et al.*, *Growth Factors* 8:245-252 (1993)) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues of the secreted VEGF-C protein lacking the signal sequence, and apparently within the first approximately 120 amino acid residues.

On the other hand, the difference between the molecular weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNA sequences are determined from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then be identified by a number of methods standard in the art, such as heteroduplex analysis of cDNA and genomic DNA, which are subsequently be characterized.



Handwritten: D2, D3  
Parklawn Drive, Rockville, MD 20852 as accession number 97231.

However, the predicted molecular weight of the mature protein product deduced from this reading-frame is 35881 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4-L mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues underlined in Fig. 10).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand subunit in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BRP3) sequence (Dignam and Case, Gene 88, 133-140, 1990), as depicted in Fig. 9A. Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand. Interestingly, at least one cysteine motif of the BRP3 type is also found in the VEGF carboxy terminal amino acid sequences.

Thus, the Flt4-L mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells. One uses antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or

PDGF/VEGF family of growth factors, as shown in Figure 10.

#### EXAMPLE 11

##### Stimulation of Flt4 autophosphorylation by the protein product of the Flt4 ligand vector

5 The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector containing the open reading frame encoding the sequence shown in Fig: 9B<sup>-</sup> (SEQ ID NO: 32) was cut out from the vector using *HindIII* and *NotI* restriction enzymes, isolated from a preparative agarose gel and ligated to the corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7  
10 vector containing the above cloned insert was transfected into 293-EBNA cells (Invitrogen) using the calcium phosphate transfection method (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). About 48 hours after transfection the medium of the transfected cells was changed to DMEM medium lacking fetal calf serum and incubated  
15 for 36 h. The thus conditioned medium was then collected, centrifuged at 5000 x g for 20 minutes, the supernatant was concentrated 5-fold using Centriprep 10 (Amicon) and used to stimulate NIH3T3<sup>373</sup> cells expressing LTRFlt4l, as in Example 4. The cells were lysed, immunoprecipitated using  
C anti-Flt4 antiserum and analysed by Western blotting using anti-phosphotyrosine antibodies.  
20

As can be seen from Fig. 11, lanes 1 and 3, the conditioned medium from two different dishes of the transfected cells stimulated Flt4 autophosphorylation in comparison with the medium from mock-transfected cells, which gave only background levels of phosphorylation of the Flt4  
25 receptor (lane 2). When the concentrated conditioned medium was pre-absorbed with 20  $\mu$ l of a <sup>slurry</sup> of Flt4EC domain coupled to Sepharose (see example 4), no phosphorylation was obtained (lane 4), showing that the activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand. Thus, these results demonstrate that the Flt4-L plasmid vector clone having an  
30 approximately 2.1 kb insert and containing the open reading frame shown in Fig. 9B is expressed into a Flt4 ligand in cells transfected with the Flt4-L expression vector clone, and thus is biologically active. The sequence encoded by that open reading frame is shown in SEQ ID NO: 33. Plasmid pFLT4-L has been deposited with the American Type Culture Collection, 12301

NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 31)  
(sense-primer corresponding to nucleotides 2179-2199 of the pcDNA1 vector).  
The amplified product was subjected to digestion with *EcoRI* (Boehringer  
Mannheim) to remove the portion of the DNA sequence amplified from the  
5 pcDNA1 vector and the resulting 153 bp fragment encoding the 5' end of the  
Flt4 ligand was labeled with [<sup>32</sup>P]-dCTP using the Klenow fragment of *E.coli*  
DNA polymerase I (Boehringer Mannheim). That fragment was used as a  
probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the  
10 radioactively labeled probe at 42 °C for 20 hours in a solution containing 50%  
formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml  
denatured salmon sperm DNA. Filters were washed twice in 1x SSC, 0.1%  
SDS for 30 minutes at room temperature, then twice for 30 minutes at 65 °C  
and exposed overnight.

15 On the basis of autoradiography, 10 positive recombinant  
bacterial colonies hybridizing with the probe were chosen from the library.  
C Plasmid DNA was purified from these colonies and analysed by *EcoRI* and  
*NotI* digestion and agarose gel electrophoresis followed by ethidium bromide  
staining. The ten plasmid clones were divided into three groups on the basis  
20 of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb,  
respectively. Inserts of plasmids from each group were sequenced using the  
T7 oligonucleotide as a primer and walking primers for subsequent sequencing  
reactions.

Sequence analysis showed that all clones contain the open  
25 reading frame encoding the NH2-terminal sequence of the Flt4 ligand.  
Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the  
signal sequence (Fig. 9A, SS). The 5' end of the 1.7 kb clone began within  
the signal sequence-encoding portion. Dideoxy sequencing was continued  
using walking primers in the downstream direction. An 1140 nucleotide  
30 portion of the sequence of the longest clone is shown in Figure 9B. As can be  
seen in that figure, after the putative signal sequence the open reading frame  
terminates in a TAA stop codon 318 amino acid residues further downstream  
C from the 32 amino acid signal sequence. When compared with sequences in  
the GenBank Database, the predicted protein product of this reading frame was  
35 found to be homologous with the predicted amino acid sequences of the

The beginning of the sequence represents the pcDNAI vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13 amino acid residues of the secreted Flt4 ligand. The cloning of the 5' end of the Flt4 cDNA, as described in the preceding two examples, is depicted schematically in Fig. 9A.

#### EXAMPLE 9

##### Amplification of the 3'-end of cDNA encoding the Flt4 ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the FLT4-L clones. The sense-strand primer 5'-ACAGAGAACAGGCCAACC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNAI vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1°C every two cycles from 72°C to 52°C, at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72°C for 3 minutes. DNA fragments of several sizes were obtained in the first amplification. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNAI vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

#### EXAMPLE 10

##### Screening the PC-3 cell cDNA library using the 5' PCR fragment of Flt4 ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTGTAGTGTGCTGCAGCGAATT-3' (antisense-strand primer, SEQ ID

PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGTGTAGTGTGCTG-3' (SEQ ID NO: 19), which is the antisense-strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20), and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21), corresponding to the T7 RNA promoter of the pcDNAI vector used for construction of the library. "Touchdown" PCR was used as disclosed in Don, *et al.*, *Nucl. Acids Res.*, 19: 4008 (1991), incorporated by reference herein. The annealing temperature of the two first cycles was 62 °C and subsequently the annealing temperature was decreased in every other cycle by 1 °C until a final temperature of 53 °C was reached, at which temperature 16 additional cycles were conducted. Annealing time was 1 minute and extension at each cycle was conducted at 72 °C for 1 minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 ul of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTGTAGTGTGCTGCAGCGAATTT-3' (SEQ ID NO: 22), an antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand, and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNAI vector. The sequences of these sense and antisense primers overlapped with the 3' ends of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72 °C to 66 °C and continuing with 18 additional cycles at 66 °C. The annealing time was 1 minute and extension at each cycle was carried out at 72 °C for 2 minutes. One major product of about 220 bp and three minor products of about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were analysed and they contained the sequence 5'-

TCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGT  
AACGGCCGCCAGTGTGGTGGAAATTCGACGAACCTCATGACTGTACTCT  
ACCCAGAATATTGGAATGTACAAGTGCAGCTAAGGCAAGGAGGC  
TGGCAACATAACAGAGAACAGGCCAACCTCAACTCAAGGACAGAAG  
AGACTATAAAATTCGCTGCAGCACACTACAAC- 3' (SEQ ID NO: 25).

lysates were centrifuged for 20 minutes at 15,000 x g. The supernatants were incubated for 2 hours on ice with 3 ul of the antiserum against the Flt4 C-terminus described in Example 2 and also in Pajusola, *et al. Oncogene* 8: 2931-2937, (1993), incorporated by reference herein.

5 After a 2 hour incubation in the presence of anti-Flt4 antiserum, protein A-Sepharose (Pharmacia) was added and incubation was continued for 45 minutes with rotation. The immunoprecipitates were washed three times with the immunoprecipitation buffer and twice with 10 mM Tris, pH7.5 before  
10 analyzed in SDS-PAGE. Polypeptides were transferred to nitrocellulose and analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine monoclonal antibodies (anti-PTyr; PY20) were purchased from Transduction Laboratories (Lexington, Kentucky). In some cases, the filters were restained with a second antibody after stripping. The stripping of  
15 the filters was done for 30 minutes at 50°C in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

As shown in Figure 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4-  
C expressing NIH<sup>3T3</sup> cells were treated with the indicated preparations of  
20 media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with the tyrosine phosphorylated, processed form of the  
25 mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of Fig. 4, see also Figure 5A). Comigration was confirmed upon restaining with anti-Flt4 antibodies as is also shown in Figure 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15 ul of  
C 30 conditioned medium <sup>were</sup> separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies. No signal was obtained (Fig. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in Figure 4, lane 1.

35 As shown in Figure 4, lane 3, stimulating activity was considerably increased when the PC-3 conditioned medium was concentrated

above (the *HindIII* site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the *SphI* site is in Flt4 cDNA). The resultant Flt4EC insert was then ligated as a *BamHI* fragment into the *BamHI* site in the pVTBac plasmid as disclosed in Tessier *et al.*, *Gene* 98: 177-183 (1991),  
5 incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection. Recombinant virus was purified, amplified and  
10 used for infection of High-Five cells (Invitrogen, San Diego, CA) using methods standard in the art. The Flt4 extracellular domain (Flt4EC) was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-  
15 terminus of the recombinant Flt4 extracellular domain.

#### EXAMPLE 4

##### Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435  
20 from the American Type Culture Collection and cultured as instructed by the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal calf serum. In order to prepare the conditioned media, confluent PC-3 cells were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the absence of fetal bovine serum. Medium was then cleared by centrifugation at 10,000 g  
25 for 20 minutes. The medium was then screened to determine its ability to induce tyrosine phosphorylation of Flt4 by exposure to NIH<sup>3T3</sup> cells which  
C had been transfected with Flt4-encoding cDNA using the pLTRFlt4l vector. C For receptor stimulation experiments, subconfluent NIH<sup>3T3</sup> cells were starved overnight in serum-free DMEM medium (GIBCO) containing 0.2% BSA. The  
30 cells were stimulated with the conditioned media for 5 minutes, washed twice with cold PBS containing 100 uM vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40 (BDH, Poole, England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 1 mM vanadate) for receptor immunoprecipitation analysis. The

*SphI* fragment of the S2.5 plasmid. The resulting vector was digested with *EcoRI* and *Clal* and ligated to a 138 bp PCR fragment amplified from the 0.6 kb *EcoRI* fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of Flt4s shown in Figure 1 of Pajusola *et al.*,

- 5 *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-  
CGGAATTCCTCATGACCCCAAC-3' (SEQ ID NO: 4) (forward, *EcoRI*  
site underlined) and 5'-CCATCGATGGATCCTACCTGAAGCCGCTTT  
CTT-3' (SEQ ID NO: 5) (reverse, *Clal* site underlined). The coding domain  
was completed by ligation of the 1.2 kb *EcoRI* fragment (base pairs 2535-3789  
10 of sequence X68203) into the above construct. The complete cDNA was  
subcloned as a *HindIII-Clal*(blunted) fragment (this *Clal* site was also included  
in the 3' primer used to construct the 3' end of the coding sequence) to the  
pLTRpoly expression vector reported in Mäkelä *et al.*, *Gene*, 118: 293-294  
(1992) (Genbank accession number X60280), incorporated by reference herein,  
15 using its *HindIII-Acc I*(blunted) restriction sites.

- The long form of Flt4 was produced by replacing the 3'-end of  
the short form as follows: The 3' region of the Flt4l cDNA was PCR-  
amplified using a gene specific and a pGEM 3Z vector specific (SP6 promoter)  
oligonucleotide 5'-ATTTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse  
20 and forward primers, respectively, and an Flt4l cDNA clone containing a 495  
bp *EcoRI* fragment extending downstream of the *EcoRI* site at nucleotide 3789  
of the Genbank X68203 sequence (the sequence downstream of this *EcoRI* site  
is deposited as the Flt4 long form 3' sequence having Genbank accession  
number S66407). The gene specific oligonucleotide contained a *BamHI*  
25 restriction site located right after the end of the coding region. The sequence  
of that (reverse primer) oligonucleotide was 5'-  
CCATCGATGGATCCCGATGCTGCTTAGTAGCTGT-3' (SEQ ID NO: 7)  
(*BamHI* site is underlined). The PCR product was digested with *EcoRI* and  
*BamHI* and transferred in frame to LTRFlt4s vector fragment from which the  
30 coding sequences downstream of the *EcoRI* site at base pair 2535 (see  
sequence X68203) had been removed by *EcoRI-BamHI* digestion. Again, the  
coding domain was completed by ligation of the 1.2 kb *EcoRI* fragment (base  
pairs 2535-3789 of sequence X68203) back into the resulting construct.



containing base pairs 56-2534 of the Flt4s into the *EcoRI* site of the pSP73 vector (Promega, Madison, WI).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAALCLRLW). Poly(A)<sup>+</sup> RNA was isolated from the HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCCTCGCTGTCCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 (Amicon Inc., Beverly, MA). Circularization was made in a total volume of 150 ul. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16°C for 16 hours. Fifteen  $\mu$ l of this reaction mix was used in a standard 100 ul PCR reaction containing 100 ng of specific primers including *SacI* and *PstI* restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95°C for 1 minute, annealing at 55°C for 2 minutes and elongation at 72°C for 4 minutes). The PCR mixture was treated sequentially with the *SacI* and *PstI* restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Zf(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an *SphI* digested PCR fragment amplified using reverse transcription-PCR of poly(A)<sup>+</sup> RNA isolated from the HEL cells using the oligonucleotides 5'-ACATG**GCATGC** CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG GACTCTGGA-3' (SEQ ID NO: 2)(forward primer, *SphI* site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATG**GCATGC** CCCGCCGGT CATCC-3' (SEQ ID NO: 3) (reverse primer, *SphI* site underlined) to the 5' end of the S2.5 fragment, thus replacing unique

and VEGF-C thus increase our understanding of the complexity of the specific and redundant positive signals for endothelial cells involved in vasculogenesis, angiogenesis, permeability and perhaps also other endothelial functions.

Also described herein is the localization of the VEGF-C gene in human chromosomes by analysis of somatic cell hybrids and fluorescence *in situ* hybridization (FISH). Southern blotting and polymerase chain reaction analysis of somatic cell hybrids and fluorescence *in situ* hybridization of metaphase chromosomes was used to assess the chromosomal localization of the VEGF-C gene. The VEGF-C gene was located on chromosome 4q34, close to the human aspartylglucosaminidase gene previously mapped to 4q34-35. The VEGF-C locus in 4q34 is a candidate target for mutations leading to vascular malformations or cardiovascular diseases. Expression studies by Northern blotting and hybridization show abundant VEGF-C expression in heart and skeletal muscle; other tissues, such as lung and kidney, also express these genes. Whereas PlGF is predominantly expressed in the placenta, the expression patterns of the three VEGFs overlap in many tissues, which suggests that they may form heterodimers and interact to exert their physiological functions.

Targeted mutagenesis leading to inactivation of the VEGF receptor loci in the mouse genome have shown that VEGFR-1 is necessary for the proper organization of endothelial cells forming the vascular endothelium, while VEGFR-2 is necessary for the generation of both endothelial and hematopoietic cells. This suggests that the four genes of the VEGF family can be targets for mutations leading to vascular malformations or cardiovascular diseases.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands and ligand-encoding nucleic acids according to the invention are shown.

#### EXAMPLE 1

##### Production of pLTR-Flt4l expression vector

Construction of the LTR-Flt4l vector is schematically shown in Figure 2. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola *et al.*, *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein,

herein further suggests that this gene product also is involved in the maintenance of the differentiated functions of the lymphatic endothelium where VEGFR-3 is expressed (Kaipainen et al., 1995). Lymphatic capillaries do not have well formed basal laminae and an interesting possibility remains that the silk-like BR3P motif is involved in producing a supramolecular structure which could regulate the availability of VEGF-C in tissues. However, as shown here, VEGF-C also activates VEGFR-2, which is abundant in proliferating endothelial cells of vascular sprouts and branching vessels of embryonic tissues, but decreased in adult tissues. Millauer et al., *Nature*, 367:576-78 (1993). These data have suggested that VEGFR-2 is a major regulator of vasculogenesis and angiogenesis. VEGF-C may thus have a unique effect in lymphatic endothelium and a more redundant function shared with VEGF in angiogenesis and possibly permeability regulation of several types of endothelia. Because VEGF-C stimulates the-VEGFR-2 and promotes endothelial migration, a utility for VEGF-C is suggested as an inducer of angiogenesis of blood and lymphatic vessels in wound healing, tissue transplantation, in eye <sup>diseases</sup> ~~diseases~~, in the formation of collateral vessels to around arterial stenoses and into injured tissues after infarction.

Taken together, these results show an increased complexity of signalling in the vascular endothelium. They reinforce the concept that when organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases in several types of functionally and morphologically distinct vessels. However, upon suitable angiogenic stimuli, endothelial cells can re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. This process of angiogenesis concurrent with tissue development and regeneration depends on the tightly controlled balance between positive and negative signals for endothelial cell proliferation, migration, differentiation and survival. Previously-identified growth factors promoting angiogenesis include the fibroblast growth factors, hepatocyte growth factor/scatter factor, PDGF and TGF- $\alpha$ . (See, e.g., Folkman, *Nature Med.* 1:27-31 (1995); Friesel and Maciag, *FASEB J.* 9:919-25 (1995); Mustonen and Alitalo, *J. Cell Biol.*, 129:895-98 (1995). However, VEGF has been the only growth factor relatively specific for endothelial cells. The newly identified factors VEGF-B

C Mutational analysis of the cysteine residues involved in the interchain disulfide bridges <sup>has</sup> have shown that, in contrast to PDGF, VEGF dimers need to be held together by these covalent interactions in order to maintain biological activity. Disulfide linking of the VEGF-C polypeptide chain was evident in the analysis of VEGF-C in nonreducing conditions.

C VEGFR-3, which thus distinguishes between VEGF and VEGF-C, is closely related <sup>in</sup> to structure to VEGFR-1 and VEGFR-2. Finnerty, *et al.*, *Oncogene*, 8:2293-98 (1993); Galland, *et al.*, *Oncogene*, 8:1233-40 (1993); Pajusola, *et al.*, *Cancer Res.*, 52:5738-43 (1992). However, the mature form of VEGFR-3 differs from the two other VEGFRs in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola, *et al.*, *Oncogene*, 9:3545-55 (1994). Another difference is that the 4.5 and 5.8 kb VEGFR-3 mRNAs encode polypeptides differing in their C-termini and apparently in their signalling properties due to the use of alternative 3' exons. Borg *et al.*, *Oncogene*, 10:973-84 (1995); Pajusola *et al.*, *Oncogene*, 8:2931-37 (1993).

Besides VEGFR-3, VEGFR-2 tyrosine kinase also is activated in response to VEGF-C. VEGFR-2 mediated signals cause striking changes in the morphology, actin reorganization and membrane ruffling of porcine aortic endothelial cells overexpressing this receptor. In these cells, VEGFR-2 also mediated ligand-induced chemotaxis and mitogenicity. Waltenberger *et al.*, *J. Biol. Chem.*, 269:26988-95 (1994). Similarly, the receptor chimera

C CSF-1R/VEGFR-3 was mitogenic when ectopically expressed in NIH3T3<sup>3T3</sup> fibroblastic cells, but not in porcine aortic endothelial cells (Pajusola *et al.*, 1994). Consistent with such results, the bovine capillary endothelial cells (BCE) which express VEGFR-2 mRNA but very little or no VEGFR-1 or VEGFR-3 mRNAs, showed enhanced migration when stimulated with VEGF-C. As shown here, light microscopy of the BCE cell cultures in collagen gel also suggested that VEGF-C stimulated the proliferation of these cells. The already existing data thus indicate that the VEGF ligands and receptors show a great specificity in their signalling, which may be cell type dependent.

C The expression pattern of the VEGFR-3 (Kaipainen *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995)) suggests that VEGF-C may function in the formation of the venous and lymphatic vascular systems during embryogenesis. Constitutive expression of VEGF-C in adult tissues shown

latter. Proteolytic processing of the VEGF-C precursor may occur at more than one cleavage site because the 32 kD molecular mass of the recombinant secreted ligand was also less than the deduced molecular mass of VEGF-C ORF without the signal peptide. By extrapolation from studies of the structure of PDGF (Heldin, *et al.*, *Growth Factors*, 8:245-52 (1993)), one can speculate that the region critical for receptor binding and activation by VEGF-C is contained within the amino-terminal first 180 or so amino acid residues of the secreted VEGF-C protein lacking the signal sequence. In fact, the region critical for receptor binding and activation by VEGF-C is believed to be contained within the first approximately 120 amino acid residues of the secreted VEGF-C protein lacking the signal sequence. Thus, the 23 kD polypeptide binding VEGFR-3 is likely to represent the VEGF-homologous domain. After biosynthesis, the nascent VEGF-C polypeptide may be glycosylated at three putative N-linked glycosylation sites identified in the deduced VEGF-C amino acid sequence.

The carboxyl terminal amino acid sequences, which increase the length of the VEGF-C polypeptide in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the <sup>Bellini</sup> ~~Bellini~~ ring 3 protein (BR3P) sequence (Dignam and Case, *Gene*, 88:133-40 (1990); Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990)). This novel C-terminal silk protein-like structural motif of VEGF-C may fold into an independent domain, which, on the basis of the considerations above, is at least partially cleaved off after biosynthesis. Interestingly, at least one cysteine motif of the BR3P type is also found in the carboxyl terminus of VEGF. In our experiments both the putative precursor and cleaved ligand were detected in the cell culture media, although processing was apparently cell-associated on the basis of the pulse-chase experiments. The determination of the amino terminal sequence of the isolated carboxyl terminal fragment will allow the identification of the proteolytic processing site. The generation of antibodies against different parts of the VEGF-C molecule will allow the exact determination of the precursor-product relationship and ratio, their cellular distribution, and the kinetics of processing and secretion.

VEGF-C has a conserved pattern of eight cysteine residues, which may participate in the formation of intra- and interchain disulfide bonds, creating an antiparallel dimeric biologically active molecule, similar to PDGF.

*Ligands of the*  
C which are ligands for the Flt4 receptor tyrosine kinase (VEGFR-3). ~~Claimed~~  
C <sup>invention</sup> ligands are members of a family of platelet-derived growth factors/vascular  
endothelial growth factors which promote mitosis and proliferation of vascular  
endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4  
5 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma  
cell line (ATCC CRL1435). When applied to a population of cells expressing  
the Flt4 receptor, ligands of the invention stimulate autophosphorylation,  
resulting in receptor activation. The invention also provides inhibitors of the  
Flt4 receptor, including antibodies directed against the ligand. A ligand  
10 according to the invention may be coexpressed as a larger precursor which is  
cleaved to produce the ligand. A coexpressed region in some cases results  
from alternative splicing of RNA of the ligand gene. Such a co-expressed  
region may be a function of the particular expression system used to obtain the  
ligand. The skilled artisan understands that in recombinant production of  
15 proteins, additional sequence may be expressed along with a functional peptide  
depending upon the particular recombinant construct used to express the  
protein, and subsequently removed to obtain the desired ligand. In some cases  
the recombinant ligand can be made lacking certain residues of the  
endogenous/natural ligand. Moreover, it is well-known in that conservative  
20 replacements may be made in a protein which do not alter the function of the  
protein. Accordingly, it is anticipated that such alterations are within the  
scope of the claims. It is intended that the precursor sequence shown in SEQ  
ID NO: 33 is capable of stimulating the Flt4 ligand without any further  
processing in a manner similar to that in which VEGF stimulates its receptor  
25 in its unprocessed form.

Results reported herein show that VEGFR-3 transmits signals  
for a novel growth factor. This conclusion is based on the specific binding of  
VEGF-C to recombinant Flt4EC (Flt4 extracellular domain) protein and the  
induction of VEGFR-3 autophosphorylation by medium from VEGF-C  
30 transfected cells. In contrast, VEGF and PlGF did not show specific binding  
to VEGFR-3 or induce its autophosphorylation.

A major part of the difference in the observed molecular mass  
of the purified and recombinant VEGF-C and the deduced molecular mass of  
the VEGF-C encoded by the VEGF-C open reading frame (ORF) may be due  
35 to proteolytic removal of sequences in the carboxyl terminal region of the

Figure 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L (VEGF-C) expression vector.

C 5 Figure 12 shows Northern blotting analysis of Flt4-L (VEGF-C) mRNA in tumor cell lines <sup>and in brain tissue</sup>.

C Figure 13A is an autoradiograph showing recombinant <sup>VEGF-C</sup> ~~VEGF-C~~ isolated following a pulse-chase experiment and electrophoresed via SDS-PAGE under reducing conditions.

C 10 Figure 13B is a photograph of polyacrylamide gel showing that recombinant <sup>VEGF-C</sup> ~~VEGF-C~~ forms are disulfide-linked in nonreducing conditions.

Figure 14A and 14B depict Western blots showing that VEGF-C stimulates autophosphorylation of VEGFR-2 (KDR) but has no effect on PDGFR- $\beta$  phosphorylation.

15 Figure 15A and 15B show that VEGF-C stimulates endothelial cell migration in a three-dimensional collagen gel assay.

Figure 16A shows the expression of VEGF-C mRNA in human adult tissues.

Figure 16B shows the expression of VEGF, VEGF-B, and VEGF-C in selected human fetal tissues.

20 Figure 17 schematically depicts the chromosomal localization of the VEGF-C gene.

Figure 18 is a Northern blot hybridization study showing the effects of hypoxia on the mRNA expression of VEGF-A, VEGF-B and VEGF-C.

25 **DETAILED DESCRIPTION OF THE INVENTION**

C ~~the cloning of a cDNA encoding this growth factor~~  
Described herein is the isolation of a novel vascular endothelial growth factor and its cloning from a cDNA library prepared from the human

prostatic adenocarcinoma cell line PC-3. The isolated cDNA encodes a protein which is proteolytically processed and secreted to cell culture medium.

30 The secreted protein, designated VEGF-C, binds to the extracellular domain of Flt4 (designated VEGFR-3) and induces tyrosine autophosphorylation of Flt4 and VEGFR-2. VEGF-C also stimulates the migration of endothelial cells in collagen gel.

The present invention also is directed to novel growth factors

oligonucleotides, and peptides which block the Flt4 receptor, all of which are intended as aspects of the invention.

#### BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a schematic diagram showing major endothelial cell  
5 receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

Figure 2 schematically depicts the construction of the pLTRFlt4l expression vector.

Figure 3 schematically depicts the construction of the  
10 baculovirus vector encoding a secreted soluble Flt4 extracellular domain (Flt4EC).

Figure 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.

*Figure 5A, 5B, and 5C show*  
C Figure 5 shows that the major tyrosyl phosphorylated  
15 polypeptide of Flt4-transfected cells stimulated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide (VEGFR-3).

Figure 6 shows Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.

*chromatographic fractions from*  
C Figure 7 shows results of gel electrophoresis of fractions from  
C 20 *the affinity purification* the Western analysis of Flt4 ligand (VEGF-C) isolated from PC-3 conditioned medium.

Figure 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand (VEGF-C), VEGF, or PIGF.

25 Figure 9A schematically depicts the cloning and analysis of the Flt4 ligand, VEGF-C. The VEGF-C coding sequence (shaded boxes) and signal sequence (ss) are depicted between 5' and 3' untranslated (ut) nucleic acid regions.

*C*  
30 Figure 9B shows the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA. The cleavage site for the putative signal peptide is indicated with a shaded triangle.

Figure 10 shows a comparison of the deduced amino acid sequences of PDGF-A, -B, two PIGF isoforms, four VEGF isoforms and Flt4 ligand (VEGF-C).

*sub C1*



In another aspect, the invention includes an antibody which is specifically reactive with polypeptides of the invention. Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells, or they may be used to block or activate the Flt4 receptor.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors *in situ*.

Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, echogenic, or radioactive agent for imaging. Other, non-radioactive labels, such as biotin and avidin, may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors are used to accelerate angiogenesis, *e.g.*, during wound healing, or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands also may be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using detectably-labeled ligand. An Flt4 ligand according to the invention also may be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention also may be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation, and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomias. For example, such inhibitors may be used to arrest metastatic growth or spread, or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense

comprises approximately amino acids 1-120 of SEQ ID NO: 33. Another preferred polypeptide of the invention comprises approximately amino acids 1-180 of SEQ ID NO: 33.

The present invention also provides a cDNA encoding a novel polypeptide, designated VEGF-C, that is structurally homologous to VEGF. VEGF-C is a ligand for the FLT4 receptor tyrosine kinase (VEGFR-3), a receptor tyrosine kinase related to VEGFR-1 and VEGFR-2 that does not bind VEGF. VEGFR-3 is expressed in venous and lymphatic endothelia of fetal tissues and predominantly in lymphatic endothelial of adult tissues. Kaipainen et al., *Cancer Res.*, 54:6571-77 (1994); Kaipainen et al., *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995).

Thus, in a preferred embodiment, the invention includes a purified and isolated nucleic acid (e.g., a DNA or an RNA) encoding an Flt4 ligand precursor. Due to the degeneracy of the genetic code, numerous such coding sequences are possible, each having in common the coding of the amino acid sequence shown in SEQ ID NO: 33. As set forth above, the invention includes polypeptides which comprise a portion of the amino acid sequence shown in SEQ ID NO: 33 and which bind the Flt4 receptor tyrosine kinase (herein designated VEGFR-3); the invention also is intended to include nucleic acids encoding these polypeptides. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase (VEGFR-3). The nucleotide sequence shown in SEQ ID NO:32 contains a preferred nucleotide sequence encoding the Flt4 ligand (VEGF-C).

The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising a DNA encoding the Flt4 ligand, and host cells comprising the vectors. Preferred vectors of the invention are capable of expressing the Flt4 ligand under the control of appropriate promoters and other control sequences. A preferred vector of the invention is plasmid pFLT4-L, having ATCC accession no. 97231.

The invention further includes a method of making polypeptides of the invention. In a preferred method, a nucleic acid or vector of the invention is expressed in a host cell, and a polypeptide of the invention is purified from the host cell or the host cell growth medium.

and VEGFR-1 also binds the related placenta growth factor (PlGF; Kd about 200 pM), while the ligands for Tie, Tek, and Flt4 have not yet been reported.

#### SUMMARY OF THE INVENTION

The present invention provides a ligand for the Flt4 receptor tyrosine kinase. Thus, the invention provides a purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in SEQ ID NO: 33. Thus, the invention includes a purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

A putative <sup>33</sup> amino acid signal peptide has been identified in the amino acid sequence shown in SEQ ID NO: 33. Thus, in a related aspect, the invention includes a purified and isolated polypeptide comprising amino acids 1-<sup>317</sup> of SEQ ID NO: 33. The Flt4 ligand precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand (herein designated VEGF-C). Thus, the invention includes a polypeptide having an amino acid sequence comprising a portion of SEQ ID NO: <sup>33</sup>8, the portion encoding a fragment capable of specifically binding to Flt4. A preferred fragment has a molecular weight of about 23 kDa as assessed by SDS-PAGE under reducing conditions. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing conditions.

Evidence suggests that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage to produce a mature, naturally-occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. Accordingly, preferred <sup>polypeptides</sup> polypeptides of the invention include polypeptides comprising amino acids 1-120, 1-121, 1-122, 1-123, 1-124 ... 1-178, 1-179, and 1-180 of SEQ ID NO: 33, wherein said polypeptides specifically bind to an Flt4 receptor tyrosine kinase. A preferred Flt4 ligand

rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only  
5 endothelial cells have been reported to proliferate in response to VEGF, and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase (VEGFR-3) is closely related  
10 in structure to the products of the VEGFR-1 and VEGFR-2 genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola *et al.*, *Cancer Res.*, 52:5738-5743 (1992). The  
C 4,5 and 5.8 kb <sup>Flt4</sup> ~~Flt4~~ mRNAs encode polypeptides which differ in their C-  
15 termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by  
20 *in situ* hybridization in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In  
C 12.5 day p.c. embryos the <sup>Flt4</sup> ~~Flt4~~ signal is observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to  
25 developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

Five endothelial cell specific receptor tyrosine kinases, Flt-1  
30 (VEGFR-1), KDR/Flk-1 (VEGFR-2), Flt4, Tie and Tek/Tie-2 have so far been described, which possess the intrinsic tyrosine kinase activity essential for signal transduction. Targeted mutations inactivating Flt-1, Flk-1, Tie and Tek in mouse embryos have indicated their essential and specific roles in  
35 vasculogenesis and angiogenesis at the molecular level. VEGFR-1 and VEGFR-2 bind VEGF with high affinity (Kd 16 pM and 760 pM, respectively)

is a dimeric glycoprotein of disulfide-linked 23 kDa subunits. Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of monocyte migration *in vitro*. Four VEGF isoforms, encoded by distinct mRNA splice variants, appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each isoform has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues remain cell surface associated and have a strong affinity for heparin.

VEGF was originally purified from several sources on the basis of its mitogenic activity toward endothelial cells, and also by its ability to induce microvascular permeability, hence it is also called vascular permeability factor (VPF). VEGF produces signals through two receptor tyrosine kinases, VEGFR-1 (FLT-1) and VEGFR-2 (KDR/Flk-1), which are expressed specifically on endothelial cells. The VEGF-related placenta growth factor (PlGF) was recently shown to bind to VEGFR-1 with high affinity. PlGF was able to enhance the growth factor activity of VEGF, but it did not stimulate endothelial cells on its own. Naturally occurring VEGF/PlGF heterodimers were nearly as potent mitogens as VEGF homodimers for endothelial cells.

The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventricular neuroectoderm produces VEGF at the capillary ingrowth stage. See Breier, *et al.*, *Development*, 114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven

Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated receptors  
5 are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, *et al.*, *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in Figure 1.

10 Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, *et al.*,  
15 *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin potentiates the stimulation of  
20 endothelial cell proliferation by angiogenic FGFs, both by protecting against denaturation and degradation and dimerizing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

Among other ligands for receptor tyrosine kinases, the platelet  
25 derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, *et al.*, *Growth Factors*, 7:261-266 (1992). Transforming growth factor  $\alpha$  (TGF $\alpha$ ) is an angiogenic factor secreted by several tumor cell types and by macrophages. Hepatocyte growth factor (HGF), the ligand of the *c-met* proto-oncogene-encoded receptor, also is  
30 strongly angiogenic.

Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain differentiated  
35 functions. The best studied of these is vascular endothelial growth factor (VEGF), a member of the PDGF family. Vascular endothelial growth factor



- 1 -

## RECEPTOR LIGAND

This is a continuation-in-part of United States Patent Application  
Serial Number 08/510,133, filed August 1, 1995, ~~by~~

## FIELD OF THE INVENTION

5 The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

## BACKGROUND OF THE INVENTION

10 Developmental growth, the remodelling and regeneration of adult tissues, as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (*in situ*) differentiating endothelial cells is termed  
15 vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis, Risau, *et al.*, *Devel. Biol.*, 125:441-450 (1988).

20 Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their  
25 tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, *et al.*, *Microvasc. Rev.*, 14:51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation,  
30 migration, differentiation, and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most significantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, *et al.*, *J. Biol. Chem.*, 267:10931-10934 (1992).

08/585845



- 52 -

# ABSTRACT

Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.




**JOINT INVENTORS**

"EXPRESS MAIL" mailing label No.  
EG473137204US.

Date of Deposit: January 12, 1996

I hereby certify that this paper (or fee) is being  
deposited with the United States Postal Service  
"EXPRESS MAIL POST OFFICE TO ADDRESSEE"  
service under 37 CFR §1.10 on the date  
indicated above and is addressed to: Assistant  
Commissioner for Patents, Washington, D.C.  
20231

  
David A. Gass

**APPLICATION FOR  
UNITED STATES LETTERS PATENT**

**SPECIFICATION**

---

**TO ALL WHOM IT MAY CONCERN:**

Be it known that we, Kari Alitalo, a citizen of Finland, residing at  
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov, a citizen of Finland,  
residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have invented a  
new and useful "RECEPTOR LIGAND", of which the following is a specification.

7. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to David A. Gass at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN,

6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: 

David A. Gass  
Reg. No: 38,153

January 12, 1996

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$375.00		\$750.00
TOTAL	16 - 20	= 0	X 11 =	\$	X 22 =	\$
INDEP.	3 - 3	= 0	X 39 =	\$	X 78 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 125 =	\$	+ 250 =	\$
Filing Fee:				\$	OR	\$750.00

B. ☐ Design Application (\$150.00/\$300.00) Filing Fee: \$ \_\_\_\_\_

C. ☐ Plant Application (\$245.00/\$490.00) Filing Fee: \$ \_\_\_\_\_

D. Other Fees

☐ Recording Assignment [Fee -- \$40.00 per assignment] \$ \_\_\_\_\_

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ \_\_\_\_\_

☐ Other \$ \_\_\_\_\_

Total Fees Enclosed **\$750.00**

6. Method of Payment of Fees

☒ Check in the amount of: **\$750.00**

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

☐ Not enclosed

3. Declaration or Oath

- ☐ Enclosed
- ☐ Executed by (check all applicable boxes)
- ☐ Inventor(s)
  - ☐ Legal representative of inventor(s)  
(37 CFR 1.42 or 1.43)
  - ☐ Joint inventor or person showing a proprietary interest on behalf of  
inventor who refused to sign or cannot be reached
    - ☐ The petition required by 37 CFR 1.47 and the statement required  
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- ☒ Not enclosed - the undersigned attorney or agent is authorized to file this  
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Declaration of Biological Deposit
- ☒ Computer-readable copy of sequence listing containing nucleotide and/or amino  
acid sequence
- ☒ Statement pursuant to 37 C.F.R. §1.821(f)
- ☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27
- ☐ Associate Power of Attorney
- ☐ Verified translation of a non-English patent application
- ☐ An assignment of the invention
- ☐ Certified copy(ies) of application(s):

COUNTRY	APPLICATION NO.	FILED

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other



08 585

**PATENT APPLICATION****IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Docket No: 28113/33072

**PATENT APPLICATION TRANSMITTAL**

**Box Patent Application**  
**Assistant Commissioner for Patents**  
**Washington, D.C. 20231**

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladimir Joukov

Title: "Receptor Ligand"

**1. Type of Application**

This new application is for a

- ☒ utility patent.  
☐ design patent.

**2. Application Papers Enclosed**

- 1 Title Page  
49 Pages of Specification (excluding Claims, Abstract & Drawings)  
2 Pages of Claims  
1 Page of Abstract  
24 Sheets of Drawings (Figs. 1 to 18)  
☐ Formal  
☒ Informal

**CERTIFICATION UNDER 37 CFR 1.10**

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on January 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473137204US.

  
David A. Gass

118 585895

FEE RECORD SHEET



4130 105

0250 teams

#2 1/2

PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: ) I hereby certify that this paper is being deposited  
) with the United States Postal Service as first  
Alitalo et al. ) class mail, postage prepaid, in an envelope  
) addressed to: Assistant Commissioner for  
Serial No.: 08/585,895 ) Patents, Washington, D.C. 20231, on this date:  
)  
Filed: January 12, 1996 ) Dated: March 28, 1996  
)  
For: Receptor Ligand ) David A. Gass  
)  
Group Art Unit: Not yet assigned ) David A. Gass  
) Registration No. 38,153  
Examiner: Not yet assigned ) Attorney for Applicant(s)

TRANSMITTAL OF EXECUTED DECLARATION

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application. No Notice to File Missing Parts has been received by the Applicants.

Also enclosed is a check in the amount of \$130.00 in payment of the fee for submission of the declaration. See 37 C.F.R. §1.16(e).

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

March 28, 1996

By: David A. Gass

David A. Gass  
Reg. No. 38,153



PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	I hereby certify that this paper is being deposited
Alitalo et al.	)	with the United States Postal Service as first
Serial No.: 08/585,895	)	class mail, postage prepaid, in an envelope
Filed: January 12, 1996	)	addressed to: Assistant Commissioner for
For: Receptor Ligand	)	Patents, Washington, D.C. 20231, on this date:
Group Art Unit: Not yet assigned	)	
Examiner: Not yet assigned	)	Dated: <u>March 28, 1996</u>
	)	<u>David A. Gass</u>
	)	Registration No. 38,153
	)	Attorney for Applicant(s)

TRANSMITTAL OF EXECUTED DECLARATION

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application. No Notice to File Missing Parts has been received by the Applicants.

Also enclosed is a check in the amount of \$130.00 in payment of the fee for submission of the declaration. See 37 C.F.R. §1.16(e).

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

March 28, 1996

By:

David A. Gass  
David A. Gass  
Reg. No: 38,153



# DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed on January 12, 1996, as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed  
☐ Yes  
☐ No

(Application Serial Number)	(Country)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
08/510,133	01 August 1995	Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**POWER OF ATTORNEY:** I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)	Trevor B. Joike (25,542)	Richard A. Schaur (30,890)	James J. Napoli (32,361)
Donald J. Bruch (19,490)	Timothy J. Venzon (26,348)	Anthony Nimmo (30,920)	Richard M. La Barge (32,254)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Christine A. Dudzik (31,245)	Jeffrey W. Smith (33,455)
Allen H. Gerstein (22,118)	Richard H. Anderson (26,526)	Kevin D. Hogg (31,879)	Douglas C. Hochstetler (33,710)
Nate F. Scarpelli (22,320)	Patrick D. Etel (26,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Donald J. Pochopien (32,167)	Robert M. Gerstein (34,824)
Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	David A. Gass (38,153)

Send correspondence to: David A. Gass

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower, 233 South Wacker Drive	Chicago, Illinois	60606-6402
Full Name of First or Sole Inventor, Kari Alitalo		Citizenship Finland		
Residence Address - Street Nyyrikintie 4A		Post Office Address - Street Same		
City (Zip) 02100 Espoo		City (Zip) Same		
State or Country FINLAND		State or Country Same		
Date March 14, 1996		Signature [Signature]		

See second page for additional inventor

See reverse for relevant rules & statutes

Second Joint Inventor, if any <u>Vladimir Joukov</u>	Citizenship <u>Finland</u> <i>Riina</i>
Residence Address - Street <u>Topeliuksenkatu 32G8</u>	Post Office Address - Street Same
City (Zip) <u>00290 Helsinki</u> <i>FI</i>	City (Zip) Same
State or Country <u>FINLAND</u>	State or Country Same
Date <u>March 14, 1996</u>	Signature <u>V. Joukov</u>



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
03/585,005	01/11/90	DAVID A. SASS	03110/00072

0272/0513  
DAVID A. SASS  
MARSHALL S. TOOLE GERSTEIN MURRAY I. BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

DATE MAILED:

03/13/98

### NOTICE TO FILE MISSING PARTS OF APPLICATION FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 1500 for large entities or \$ 150 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 136.00.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ \_\_\_\_\_ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ \_\_\_\_\_ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
3. ☒ The oath or declaration:  
☒ is missing.  
☐ does not cover the newly submitted items.  

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:  
\_\_\_\_\_. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ \_\_\_\_\_ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ \_\_\_\_\_ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

**A copy of this notice MUST be returned with the response.**





#130 122 0300  
5/16/96  
PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.	)	"EXPRESS MAIL"
Serial No: 08/585,895	)	Mailing label No. EM118660766US
Filed: January 16, 1996	)	Date of Deposit:
Title: RECEPTOR LIGAND	)	May 24, 1996
Group Art Unit: Not yet assigned	)	I hereby certify that this paper (or fee)
Examiner: Not yet assigned	)	is being deposited with the United
	)	States Postal Service "EXPRESS
	)	MAIL POST OFFICE TO ADDRESSEE"
	)	service under 37 CFR §1.10 on the
	)	date indicated above and is addressed
	)	to the Assistant Commissioner for
	)	Patents,
	)	Washington, D.C., 20231.
	)	<u>Mark Bonadonna</u>
	)	Mark Bonadonna

Petition to Accord a Filing Date of January 12, 1996,  
Pursuant to 37 C.F.R. §§ 1.6, 1.10,  
and 1.53, and M.P.E.P. §506.02

or, in the alternative,

Petition to Suspend Rules Pursuant to  
35 U.S.C. §21 and 37 C.F.R. § 1.183  
to Accord Filing Date of January 12, 1996

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attn: Special Program Law Office

Dear Sir:

The Applicants request that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. The application was filed in accordance with 37 C.F.R. §1.10 and 1.53(a) on January 12, 1996.

If this request is denied, then the Applicants hereby petition to accord a filing date of January 12, 1996, pursuant to 37 C.F.R. §§ 1.6, 1.10, and 1.53, and M.P.E.P. §506.02. In the alternative, the Applicants petition to suspend the rules pursuant to 35 U.S.C. §21 and 37 C.F.R. §1.183, to Accord Filing Date of January 12, 1996.

I. **Petition for Review of Refusal to Accord Filing Date Pursuant to 37 C.F.R. §§ 1.6, 1.10, 1.17(h) and 1.53, and M.P.E.P. §506.02**

A. **Statement of Facts**

The above-identified patent application was filed in accordance with 37 C.F.R. §1.53(a) on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. Copies of the Applicants' transmittal letter, specification cover sheet, and express mail mailing receipt are submitted herewith as Exhibits 1, 2, and 3, respectively. The transmittal letter and specification cover sheet contain certificates of mailing in accordance with 37 C.F.R. §1.10, dated January 12, 1996.

On May 15, 1996, the Patent and Trademark Office mailed a Notice to File Missing Parts of Application -- Filing Date Granted. (Exhibit 4.) However, the filing date on the Notice was Tuesday, January 16, 1996, instead of January 12, 1996.

B. **Argument**

The Patent and Trademark Office has not identified any defects in the application or certificates of mailing dated Friday, January 12, 1996. Friday, January 12, 1996, was not a Saturday, Sunday, or Federal holiday.<sup>1</sup> Accordingly, under the rules promulgated by the Commissioner, the above-identified application properly should be considered as having been filed on January 12, 1996. See 37 C.F.R. §1.10 (a). Correction of the filing date to January 12, 1996, is respectfully requested.

II. **Conditional Petition to Suspend Rules to Accord a Filing Date of January 12, 1996**

If the foregoing petition is denied on the grounds that the Commissioner declared January 12, 1996, to be a "Federal holiday" as that term is used in 37 C.F.R. §1.10, then the Applicants hereby petition the Commissioner to suspend the rules pursuant to 37 C.F.R. §1.183, and to accord the present application a filing date of January 12, 1996. This petition has been filed following a telephone interview between the undersigned attorney and Examiner

---

<sup>1</sup> As set forth in M.P.E.P. §710.05, the Federal holidays are New Year Day, Martin Luther King's Birthday, Washington's Birthday, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving Day, Christmas Day, and Inauguration Day.

Nguyen concerning this matter on May 20, 1996, which interview the Applicants acknowledge with thanks.

**A. Statement of Facts**

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal Holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10, as explained in detail in Section I above. (See Exhibits 1-3.) However, the application was accorded a filing date of January 16, 1996. (See Exhibit 4.)

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

**B. Argument**

The Applicants' reliance on the Express Mail procedures of 37 C.F.R. §1.10 for the present application, to obtain a filing date of January 12, 1996, was reasonable, because January 12, 1996, was not a Saturday, Sunday, or recognized Federal holiday. If January 12 was deemed a "Federal holiday" by the Commissioner, it was so deemed, without advance warning to the Applicants, due to an unscheduled and unforeseeable event -- adverse weather conditions in the District of Columbia. An apparent purpose of deeming such days to be "Federal holidays" is to protect applicants' patent rights, by allowing for the timely filing of papers or fees on the next succeeding business day. See M.P.E.P. §510. However, the effect of denying the present Applicants a filing date of January 12, 1996, on the grounds of an unscheduled, weather-related "Federal holiday" being declared, may be to destroy the present Applicants' valuable patent rights in foreign countries. The Applicants submit that the foregoing unforeseeable circumstances comprise an extraordinary situation, and that justice requires the suspension of rules to accord the present application a filing date of January 12,

1996, to preserve the Applicants' foreign patent rights. The granting of such a filing date is not believed to contravene any requirements of the patent statutes. In fact, the granting of the January 12, 1996, filing date is submitted to be in complete harmony with the purpose and intent of 35 U.S.C. §21 and 37 C.F.R. §§1.6 and 1.10.

#### SUMMARY

The Applicants respectfully request and petition that the present application be accorded a filing date of January 12, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: MAY 24, 1996



David A. Gass  
Registration No. 38,153

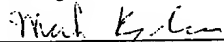
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300





**PATENT**  
**28113/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Alitalo et al.	)	<b>"EXPRESS MAIL"</b>
Serial No: 08/585,895	)	Mailing label No. EM118660766US
Filed: January 16, 1996	)	Date of Deposit:
Title: RECEPTOR LIGAND	)	May 24, 1996
Group Art Unit: Not yet assigned	)	I hereby certify that this paper (or fee)
Examiner: Not yet assigned	)	is being deposited with the United
	)	States Postal Service "EXPRESS
	)	MAIL POST OFFICE TO ADDRESSEE"
	)	service under 37 CFR §1.10 on the
	)	date indicated above and is addressed
	)	to the Assistant Commissioner for
	)	Patents,
	)	Washington, D.C., 20231.
	)	
	)	Mark Bonadonna

Petition to Accord a Filing Date of January 12, 1996,  
Pursuant to 37 C.F.R. §§ 1.6, 1.10,  
and 1.53, and M.P.E.P. §506.02

or, in the alternative,

Petition to Suspend Rules Pursuant to  
35 U.S.C. §21 and 37 C.F.R. § 1.183  
to Accord Filing Date of January 12, 1996

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attn: Special Program Law Office

Dear Sir:

The Applicants request that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. The application was filed in accordance with 37 C.F.R. §1.10 and 1.53(a) on January 12, 1996.

If this request is denied, then the Applicants hereby petition to accord a filing date of January 12, 1996, pursuant to 37 C.F.R. §§ 1.6, 1.10, and 1.53, and M.P.E.P. §506.02. In the alternative, the Applicants petition to suspend the rules pursuant to 35 U.S.C. §21 and 37 C.F.R. § 1.183, to Accord Filing Date of January 12, 1996.

I. Petition for Review of Refusal to Accord Filing Date Pursuant to 37 C.F.R. §§ 1.6, 1.10, 1.17(h) and 1.53, and M.P.E.P. §506.02

A. Statement of Facts

The above-identified patent application was filed in accordance with 37 C.F.R. §1.53(a) on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. Copies of the Applicants' transmittal letter, specification cover sheet, and express mail mailing receipt are submitted herewith as Exhibits 1, 2, and 3, respectively. The transmittal letter and specification cover sheet contain certificates of mailing in accordance with 37 C.F.R. §1.10, dated January 12, 1996.

On May 15, 1996, the Patent and Trademark Office mailed a Notice to File Missing Parts of Application -- Filing Date Granted. (Exhibit 4.) However, the filing date on the Notice was Tuesday, January 16, 1996, instead of January 12, 1996.

B. Argument

The Patent and Trademark Office has not identified any defects in the application or certificates of mailing dated Friday, January 12, 1996. Friday, January 12, 1996, was not a Saturday, Sunday, or Federal holiday.<sup>1</sup> Accordingly, under the rules promulgated by the Commissioner, the above-identified application properly should be considered as having been filed on January 12, 1996. See 37 C.F.R. §1.10 (a). Correction of the filing date to January 12, 1996, is respectfully requested.

II. Conditional Petition to Suspend Rules to Accord a Filing Date of January 12, 1996

If the foregoing petition is denied on the grounds that the Commissioner declared January 12, 1996, to be a "Federal holiday" as that term is used in 37 C.F.R. §1.10, then the Applicants hereby petition the Commissioner to suspend the rules pursuant to 37 C.F.R. §1.183, and to accord the present application a filing date of January 12, 1996. This petition has been filed following a telephone interview between the undersigned attorney and Examiner

<sup>1</sup> As set forth in M.P.E.P. §710.05, the Federal holidays are New Year Day, Martin Luther King's Birthday, Washington's Birthday, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving Day, Christmas Day, and Inauguration Day.

Nguyen concerning this matter on May 20, 1996, which interview the Applicants acknowledge with thanks.

#### A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal Holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10, as explained in detail in Section I above. (See Exhibits 1-3.) However, the application was accorded a filing date of January 16, 1996. (See Exhibit 4.)

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

#### B. Argument

The Applicants' reliance on the Express Mail procedures of 37 C.F.R. §1.10 for the present application, to obtain a filing date of January 12, 1996, was reasonable, because January 12, 1996, was not a Saturday, Sunday, or recognized Federal holiday. If January 12 was deemed a "Federal holiday" by the Commissioner, it was so deemed, without advance warning to the Applicants, due to an unscheduled and unforeseeable event -- adverse weather conditions in the District of Columbia. An apparent purpose of deeming such days to be "Federal holidays" is to protect applicants' patent rights, by allowing for the timely filing of papers or fees on the next succeeding business day. See M.P.E.P. §510. However, the effect of denying the present Applicants a filing date of January 12, 1996, on the grounds of an unscheduled, weather-related "Federal holiday" being declared, may be to destroy the present Applicants' valuable patent rights in foreign countries. The Applicants submit that the foregoing unforeseeable circumstances comprise an extraordinary situation, and that justice requires the suspension of rules to accord the present application a filing date of January 12,

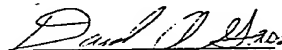
1996, to preserve the Applicants' foreign patent rights. The granting of such a filing date is not believed to contravene any requirements of the patent statutes. In fact, the granting of the January 12, 1996, filing date is submitted to be in complete harmony with the purpose and intent of 35 U.S.C. §21 and 37 C.F.R. §§1.6 and 1.10.

#### SUMMARY

The Applicants respectfully request and petition that the present application be accorded a filing date of January 12, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: MAY 24, 1996



David A. Gass  
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300



Exhibit 1

**PATENT APPLICATION**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Docket No: 28113/33072

**PATENT APPLICATION TRANSMITTAL**

*Box Patent Application  
Assistant Commissioner for Patents  
Washington, D.C. 20231*

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladimir Joukov

Title: "Receptor Ligand"

**1. Type of Application**

This new application is for a

- ☒ utility patent.  
☐ design patent.

**2. Application Papers Enclosed**

- |                                     |  |
|-------------------------------------|--|
| 1                                   | Title Page   |
| 49                                  | Pages of Specification (excluding Claims, Abstract & Drawings) |
| 2                                   | Pages of Claims  |
| 1                                   | Page of Abstract   |
| 24                                  | Sheets of Drawings (Figs. 1 to 18)                             |
| <input type="checkbox"/>            | Formal   |
| <input checked="" type="checkbox"/> | Informal   |

---

**CERTIFICATION UNDER 37 CFR 1.10**

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on January 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473137204US.

  
David A. Gass

3. Declaration or Oath

- ☐ Enclosed
- ☐ Executed by (check all applicable boxes)
- ☐ Inventor(s)
- ☐ Legal representative of inventor(s)  
(37 CFR 1.42 or 1.43)
- ☐ Joint inventor or person showing a proprietary interest on behalf of  
inventor who refused to sign or cannot be reached
- ☐ The petition required by 37 CFR 1.47 and the statement required  
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- ☒ Not enclosed - the undersigned attorney or agent is authorized to file this  
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Declaration of Biological Deposit
- ☒ Computer-readable copy of sequence listing containing nucleotide and/or amino  
acid sequence
- ☒ Statement pursuant to 37 C.F.R. §1.821(f)
- ☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27
- ☐ Associate Power of Attorney
- ☐ Verified translation of a non-English patent application
- ☐ An assignment of the invention
- ☐ Certified copy(ies) of application(s):

COUNTRY	APPLICATION NO.	FILED

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)					
			SMALL ENTITY		OTHER THAN A SMALL ENTITY
	NO. FILED	NO. EXTRA	RATE	FEE	
BASIC FEE				\$375.00	\$750.00
TOTAL	16 - 20	= 0	X 11 =	\$	X 22 = \$
INDEP.	3 - 3	= 0	X 39 =	\$	X 78 = \$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 125 =	\$	+ 250 =
Filing Fee:				\$	OR \$750.00

- B. ☐ Design Application (\$150.00/\$300.00) Filing Fee: \$ \_\_\_\_\_
- C. ☐ Plant Application (\$245.00/\$490.00) Filing Fee: \$ \_\_\_\_\_

D. Other Fees

- ☐ Recording Assignment [Fee -- \$40.00 per assignment] \$ \_\_\_\_\_
- ☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ \_\_\_\_\_
- ☐ Other \$ \_\_\_\_\_

Total Fees Enclosed \$750.00

6. Method of Payment of Fees

- ☒ Check in the amount of: \$750.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.
- ☐ Not enclosed

7. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to David A. Gass at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By:



David A. Gass  
Reg. No: 38,153

January 12, 1996






JOINT INVENTORS

Exhibit 2

"EXPRESS MAIL" mailing label No.  
EG473137204US.

Date of Deposit: January 12, 1996

I hereby certify that this paper (or fee) is being  
deposited with the United States Postal Service  
"EXPRESS MAIL POST OFFICE TO ADDRESSEE"  
service under 37 CFR §1.10 on the date  
indicated above and is addressed to: Assistant  
Commissioner for Patents, Washington, D.C.  
20231

  
David A. Gass

APPLICATION FOR  
UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo, a citizen of Finland, residing at  
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov, a citizen of Finland,  
residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have invented a  
new and useful "RECEPTOR LIGAND", of which the following is a specification:



Exhibit 3

POST OFFICE TO ADDRESSEE		EXPRESS MAIL		EG473137204US	
RIGHT (POSTAL USE ONLY)					
Origin (City, State, ZIP)		Date of Delivery (City, State, ZIP)		Postage	
Chicago, IL 60606		May 24, 1996		15.00	
Time In		Time Out		Weight	
10:00 AM		1:00 PM		1.00 lb	
Weight		Int'l Alpha Country Code		Total Postage & Fees	
1.00 lb		US		15.00	
No Delivery		Acceptance		Signature	
<input type="checkbox"/> Weekend <input type="checkbox"/> Holiday		<input checked="" type="checkbox"/> Clerk Initials		15.00	
CUSTOMER USE ONLY					
METHOD OF PAYMENT:					
Express Mail Corporate Acct. No.					
Federal Agency Acct. No. or Postal Service Acct. No.					
FROM: (PLEASE PRINT)			TO: (PLEASE PRINT)		
28113/38072			Assistant Commissioner		
DAVID A. GASS			for Patents		
MARSHALL, O'TOOLE, GERSTEIN,			Washington, D.C. 20231		
MURRAY & BORUN					
6300 SEARS TOWER					
233 SOUTH WACKER DRIVE					
CHICAGO, ILLINOIS 60606-6402					
Box Patent Application					

EL 11-8 11/93

For Pickup or Tracking Call 1-800-222-1811



Exhibit 4  
UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
08/535,895	01/16/96	ALITALO	K 28113/33072

0272/0515  
DAVID A GASS  
MARSHALL O'TOOLE GERSTEIN MURRAY & BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6400

RECEIVED  
MAY 20 1996

DATE MAILED: 05/15/96

NOTICE TO FILE MISSING PARTS OF APPLICATION  
FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130.00 for large entities or \$ 65.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 130.00.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ \_\_\_\_\_ to complete the basic filing fee.
- ☐ Additional claim fees of \$ \_\_\_\_\_ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- ☒ The oath or declaration:  
☒ is missing.  
☐ does not cover the newly submitted items.

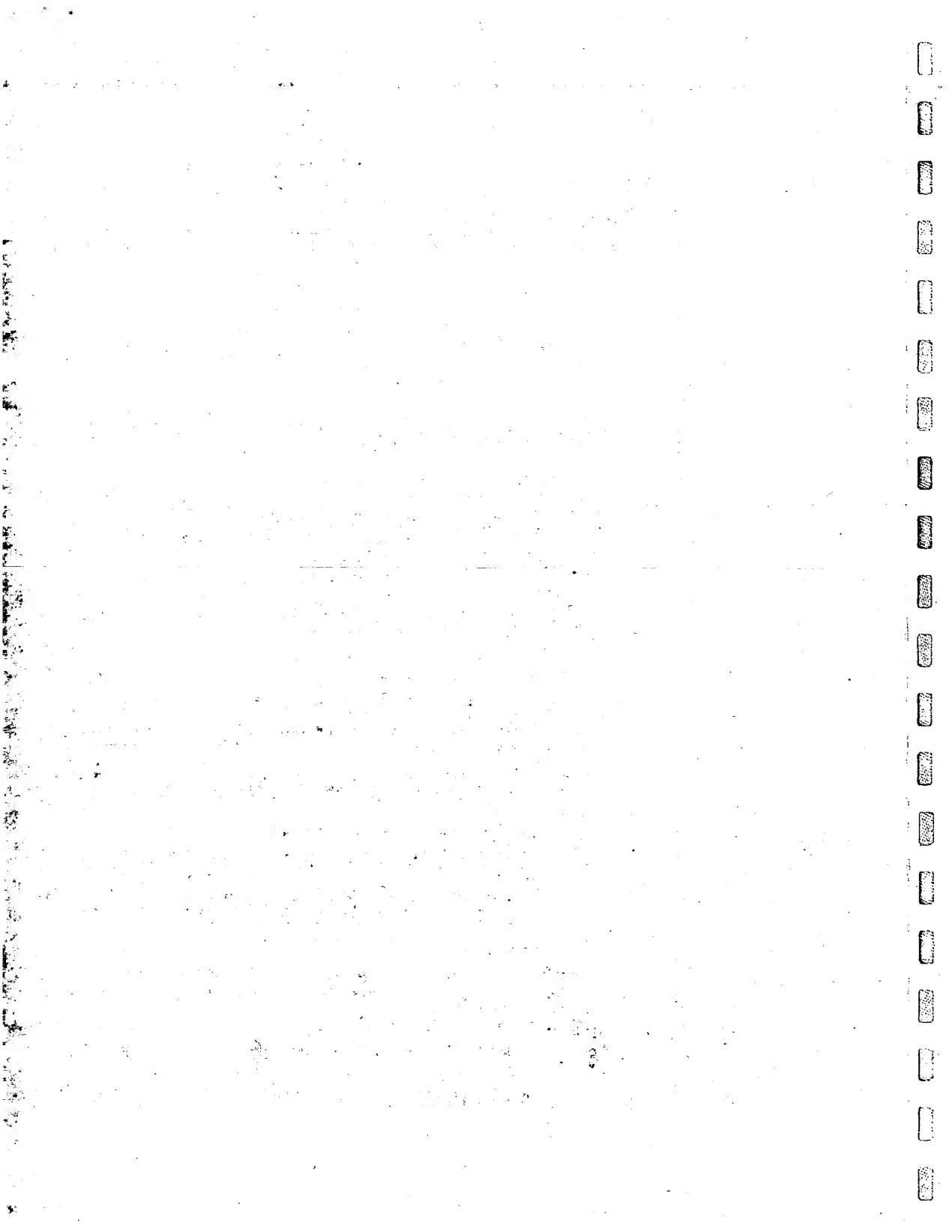
An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.

- ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:  
\_\_\_\_\_. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
- ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ \_\_\_\_\_ under 37 CFR 1.170(k), unless this fee has already been paid.
- ☐ A \$ \_\_\_\_\_ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
- ☐ Your filing receipt was mailed in error because your check was returned without payment.
- ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
- ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice **MUST** be returned with the response.

ATTORNEY'S/APPLICANTS COPY





130-122 B

PATENT # K  
28113/33072

NOTE PA  
NUMBER  
FIVE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 16, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

Examiner: Not yet assigned

"EXPRESS MAIL"

Mailing label No.: EM118663762US

Date of Deposit:  
June 25, 1996

I hereby certify that this paper (or fee) is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 CFR § 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C., 20231.

Mark Bonadonna  
Mark Bonadonna

Petition to Expedite Handling of Earlier Filed Petition  
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attn: Petitions Office

Dear Sir:

On May 24, 1996, the Applicants filed a petition (the "Filing Date Petition") requesting that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. For the reasons set forth below, the Applicants request that the Filing Date Petition receive expedited handling. This request is accompanied by a check for \$130 in payment of the petition fee.

The Applicants request expedited handling for the purposes of international filings which will claim priority from the present application.

**A. Statement of Facts**

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. However, the application was accorded a filing date of the following Tuesday, January 16, 1996. On May 24, 1996, promptly after receiving a Notice to File Missing Parts dated May 15, 1996, the Applicants filed the Filing Date Petition, setting forth reasons and facts why the present application should be accorded a filing date of January 12, 1996.

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

The present application is a continuation-in-part of U.S. Patent Application Serial No. 08/510,133, filed August 1, 1995. To obtain the priority benefit of both the parent application and the present application under the Paris Convention, the Applicants intend to file at least one foreign application on or before August 1, 1996.

**B. Argument**

The Applicants request an expedited decision on the Filing Date Petition to permit the Applicants to make an informed and accurate foreign filing that claims priority from the above-identified application. Specifically, the Applicants request that the Patent Office render its decision on the Filing Date Petition substantially in advance of the August 1, 1996.

First, the decision on the Filing Date Petition will impact the accuracy of the Applicants' foreign filings. It is important to identify priority documents in foreign filings with particularity, e.g., by serial number and filing date. A decision

on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

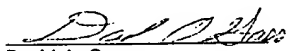
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

#### SUMMARY

The Applicants respectfully request and petition that their earlier-filed petition (to accord the present application a filing date of January 12, 1996) be handled expeditiously, such that a decision is rendered substantially in advance of August 1, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: 25 June 1996

  
David A. Gass  
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300



PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 16, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

Examiner: Not yet assigned

"EXPRESS MAIL"

Mailing label No.: EM118663762US

Date of Deposit:  
June 25, 1996

I hereby certify that this paper (or fee)  
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service under 37 CFR §1.10 on the  
date indicated above and is addressed  
to the Assistant Commissioner for  
Patents,  
Washington, D.C., 20231.

*Mark Bonadonna*  
Mark Bonadonna

Petition to Expedite Handling of Earlier Filed Petition  
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attn: Petitions Office

Dear Sir:

On May 24, 1996, the Applicants filed a petition (the "Filing Date Petition") requesting that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. For the reasons set forth below, the Applicants request that the Filing Date Petition receive expedited handling. This request is accompanied by a check for \$130 in payment of the petition fee.



The Applicants request expedited handling for the purposes of international filings which will claim priority from the present application.

**A. Statement of Facts**

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. However, the application was accorded a filing date of the following Tuesday, January 16, 1996. On May 24, 1996, promptly after receiving a Notice to File Missing Parts dated May 15, 1996, the Applicants filed the Filing Date Petition, setting forth reasons and facts why the present application should be accorded a filing date of January 12, 1996.

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

The present application is a continuation-in-part of U.S. Patent Application Serial No. 08/510,133, filed August 1, 1995. To obtain the priority benefit of both the parent application and the present application under the Paris Convention, the Applicants intend to file at least one foreign application on or before August 1, 1996.

**B. Argument**

The Applicants request an expedited decision on the Filing Date Petition to permit the Applicants to make an informed and accurate foreign filing that claims priority from the above-identified application. Specifically, the Applicants request that the Patent Office render its decision on the Filing Date Petition substantially in advance of the August 1, 1996.

First, the decision on the Filing Date Petition will impact the accuracy of the Applicants' foreign filings. It is important to identify priority documents in foreign filings with particularity, e.g., by serial number and filing date. A decision

on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

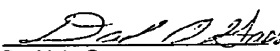
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

#### SUMMARY

The Applicants respectfully request and petition that their earlier-filed petition (to accord the present application a filing date of January 12, 1996) be handled expeditiously, such that a decision is rendered substantially in advance of August 1, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

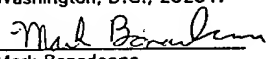
Dated: 25 June 1996

  
David A. Gass  
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

**PATENT**  
**28113/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Alitalo et al.	)	"EXPRESS MAIL"
	)	Mailing label No.: EM118663762US
Serial No: 08/585,895	)	
	)	Date of Deposit:
Filed: January 16, 1996	)	June 25, 1996
	)	
Title: RECEPTOR LIGAND	)	I hereby certify that this paper (or fee)
	)	is being deposited with the United
Group Art Unit: Not yet assigned	)	States Postal Service "EXPRESS
	)	MAIL POST OFFICE TO ADDRESSEE"
Examiner: Not yet assigned	)	service under 37 CFR §1.10 on the
	)	date indicated above and is addressed
	)	to the Assistant Commissioner for
	)	Patents,
	)	Washington, D.C., 20231.
	)	
	)	Mark Bonadonna

Petition to Expedite Handling of Earlier Filed Petition  
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents  
Washington, D.C. 20231

**FAX RECEIVED**

JUL 17 1996

**PATENT OFFICE**

Attn: Petitions Office

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**B. Argument**

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on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

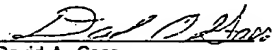
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

#### SUMMARY

The Applicants respectfully request and petition that their earlier-filed petition (to accord the present application a filing date of January 12, 1996) be handled expeditiously, such that a decision is rendered substantially in advance of August 1, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: 25 June 1996

  
David A. Gass  
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

\*\*\*\*\*  
\*\*\* ACTIVITY REPORT \*\*\*  
\*\*\*\*\*

RECEPTION OK

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MARSHALL, O'TOOLE

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MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN

RICHARD H. ANDERSON  
MICHAEL F. BORUN  
DONALD J. BRYOT  
MADELINE HENRICKS DEVEREUX  
CHRISTINE A. DUDZIK  
PATRICK D. EITEL  
ALLEN H. GERSTEIN  
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NATE F. SCARPELLI  
CYNTHIA L. SCHALLER  
RICHARD A. SCHURER  
JEFFREY S. SHARP  
ALVIN D. SHULMAN  
JEFFREY W. SMITH  
TIMOTHY J. VEZEAU  
KARL A. VICK  
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SCOTT M. GETTLESON  
MICHAEL R. GRAHAM  
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DANIELLE M. JOHNSTON  
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STEPHEN M. MILLER  
UMSIEN RINLAURES, M.D.  
DOUGLAS H. SEGEL  
DOUGLAS J. SUN, PH.D.

July 17, 1996

FACSIMILE  
TRANSMISSION SHEET

BY TELEPHONE  
JOHN H. COULT

REGISTERED PATENT AGENTS  
GRETA E. NOLAND  
JOSEPH A. WILLIAMS, JR., PH.D.

TO: Tim Heightbrink

CLIENT NO.: 28113

FAX NO.: (703) 308-6916

MATTER NO.: 33072

FROM: David A. Gass

COUNTRY CODE: US

FAX NO.: (312) 474-0448

PAGES (INCLUDING THIS PAGE): 4

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JUL 17 1996

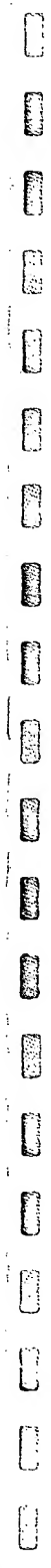
MESSAGE: \_\_\_\_\_

PETITIONS OFFICE

Please contact \_\_\_\_\_ at 474-6300 if you do  
not receive all of the pages in good condition.

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UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Paper No. 7

David A. Gass  
Marshall, O'Toole, Gerstein,  
Murray & Borun  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402

**COPY MAILED**

**JUL 22 1996**

**OFFICE OF PETITIONS  
AND PATENTS**

In re Application of :  
Alitalo et al. :  
Application No. 08/585,895 :  
Filed: January 12, 1996 :  
Attorney Docket No. 28113/33072 :  
DECISION GRANTING PETITION

This is a decision on the petition filed May 24, 1996 made special by the petition filed by "Express Mail" June 25, 1996 (copy of the July 25, 1996 petition sent July 17, 1996, by facsimile transmission in response to a telephone communication by Tim Heitbrink, of the Office of Petitions), requesting that the above-identified application be accorded a filing date of January 12, 1996.

The application, which is a continuation-in-part application under 37 CFR 1.53, was deposited in Express Mail service on January 12, 1996, which was a Friday. The Express Mail label number was placed on the papers. However, Federal and District of Columbia government offices, including the Patent and Trademark Office (Office), were officially closed for the entire day on January 12, 1996, as a result of adverse weather conditions. Under such conditions, the Office considers that day as a "federal holiday within the District of Columbia" under 35 U.S.C. 21. See 1183 OG 60 and notice entitled "Filing Of Papers During Unscheduled Closings Of The Patent and Trademark Office", originally published at 1097 OG 53, reprinted at 1158 OG 8 (copies enclosed).

In accordance with Office procedure, the present application was accorded a filing date of Tuesday, January 16, 1996, the next business day following the date of deposit in Express Mail service. The fact that no papers are received or stamped on Saturdays, Sundays or Federal holidays within the District of Columbia and the handling of Express Mail in such cases is clearly set out in 37 CFR 1.6(a) and 1.10(a).

Of course, "in an extraordinary situation, where justice requires" the Commissioner may waive or suspend the rules and accord this application a January 12, 1996 filing date pursuant

Application No. 08/585,895

Page 2

to 37 CFR 1.183. In this case, petitioners request waiver of the rules under 37 CFR 1.183 based on the need to protect applicants' patent rights in foreign countries.

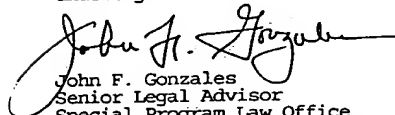
Under the circumstances of this particular case, it is deemed appropriate to waive the rules pursuant to 37 CFR 1.183 in order to accord the application a filing date of January 12, 1996.

The petition under 37 CFR 1.183 is granted.

Receipt is acknowledged of the combined declaration and power of attorney filed April 1, 1996.

The application is being returned to Application Processing Division for further processing with a filing date of January 12, 1996.

Any inquiries related to this decision should be directed to Tim Heitbrink at (703) 308-6713, or if not available, to the undersigned at (703) 305-9282.



John F. Gonzales  
Senior Legal Advisor  
Special Program Law Office  
Office of the Deputy Assistant Commissioner  
for Patent Policy and Projects

twh

Enclosure: 1183 OG 60  
1158 OG 8



**PATENT**  
**28113/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

Examiner: Not yet assigned

I hereby certify that this paper is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C., 20231 on this date:

Date: August 12, 1996

David A. Gass  
David A. Gass  
Registration No. 38,153  
Attorney for Applicants

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary Amendment prior to examination of the above-identified application on the merits by the Patent and Trademark Office.

**AMENDMENTS**

**In the Specification:**

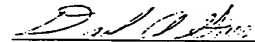
At page 1, line 3, after "August 1, 1995.", please insert -- This application is also a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994.--

REMARKS

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

Respectfully submitted,

Dated: August 11, 1988



David A. Gass  
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, Kari	)	I hereby certify that this paper and
and Joukov, Vladimir	)	the documents referred to as enclosed
Serial No.: 08/585,895	)	herewith are being deposited with the
	)	United States Postal Service as First
	)	Class Mail, postage prepaid, in an
	)	envelope addressed to: Assistant
Filed: January 12, 1996	)	Commissioner for Patents,
	)	Washington, DC 20231, on this date:
For: "Receptor Ligand"	)	
	)	October 14, 1996
Group Art Unit: 1806	)	<u>David A. Gass</u>
	)	David A. Gass
	)	Reg. No.: 38,153
Examiner: To be determined	)	Attorney for Applicants

**INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

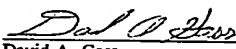
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

October 14, 1996

By:

David A. Gass  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, Kari	)	I hereby certify that this paper and
and Joukov, Vladimir	)	the documents referred to as enclosed
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Filed: January 12, 1996	)	United States Postal Service as First
For: "Receptor Ligand"	)	Class Mail, postage prepaid, in an
Group Art Unit: 1806	)	envelope addressed to: Assistant
Examiner: To be determined	)	Commissioner for Patents,
	)	Washington, DC 20231, on this date:
	)	October 14, 1996
	)	
	)	David A. Gass
	)	Reg. No.: 38,153
	)	Attorney for Applicants

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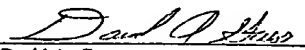
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MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

October 14, 1996

By:

  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
02/585,895	01/12/96	ACITHELO	K 28113/33672

18M2 1123  
MARSHALL O'TOOLE GERSTEIN KURWAY & BORUM  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

EXAMINER	
LOTHROP, B	
ART UNIT	PAPER NUMBER
1801	6
DATE MAILED: 11/23/96	

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☐ Responsive to communication(s) filed on \_\_\_\_\_
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 0 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(s).

Disposition of Claims

- ☒ Claim(s) 1-16 is/are pending in the application.
- Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- ☐ Claim(s) \_\_\_\_\_ is/are rejected.
- ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- ☒ Claims 1-16 are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). \_\_\_\_\_
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Serial Number: 08/585895  
Art Unit: 1801

2

### DETAILED ACTION

#### *Election/Restriction*

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:
  - I. Claims 1, 2, 8-10, 12, and 16, drawn to a ligand for the Flt4 receptor and compositions comprising the same, classified in class 530, subclass 399.
  - II. Claims 3-7, 11, and 13, drawn to nucleic acids encoding a ligand for the Flt4 receptor and vectors and host cells comprising the same, classified in class 536, subclass 23.51.
  - III. Claims 14-15, drawn to an antibody specifically reactive to a ligand for the Flt4 receptor, classified in class 530, subclass 387.1.
2. The inventions are distinct, each from the other because of the following reasons:
3. The protein of Group I is a patentably distinct chemical species from the nucleic acids of Group II, although related as the nucleic acids encode the protein. The protein can be made without recourse to the nucleic acids by the materially distinct process of biochemical purification from tissue or serum, and the nucleic acids have separate utility as probes for screening expression libraries.
4. The protein of Group I is a patentably distinct chemical species from the antibody of Group III, although related as the antibody can bind the protein. The antibody can cross-react with other proteins, and other antibodies can cross-react with the protein. The protein can be



Serial Number: 08/585895  
Art Unit: 1801

3

made without recourse to the antibody by the materially distinct process of biochemical purification from tissue or serum, and the protein has separate utility as a therapeutic agent.

5. The antibodies of Group III are patentably distinct from the nucleic acids of Group II, although related as the antibodies may be raised against proteins encoded by the nucleic acids. The inventions have distinct chemical compositions and distinct functions. The nucleic acids are not required to make the antibodies, which may be raised against proteins made without recombinant expression. The nucleic acids have separate utility as probes, for example.

6. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

7. A telephone call was made to David Gass on 14 November 1996 to request an oral election to the above restriction requirement, but did not result in an election being made.

Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

8. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(h).

Serial Number: 08/585895  
Art Unit: 1801

4

*Conclusion*

9. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Vasu Jagannathan, can be reached at (703) 306-2777. The FAX number for Art Unit 1801 is (703) 305-7401.

An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.

Art Unit 1801

*Vasu Jagannathan*  
SPE  
AU 1801

1806  
038° T/8  
PATENT #  
28113/33072 G.  
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prel

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.	)	I hereby certify that this paper is
Serial No: 08/585,895	)	being deposited with the United
Filed: January 12, 1996	)	States Postal Service with sufficient
Title: RECEPTOR LIGAND	)	postage as first class mail in an
Group Art Unit: Not yet assigned	)	envelope addressed to: Assistant
Examiner: Not yet assigned	)	Commissioner for Patents,
	)	Washington, D.C., 20231 on this
	)	date:
	)	Date: <u>August 12, 1996</u>
	)	<u>David A. Gass</u>
	)	David A. Gass
	)	Registration No. 38,153
	)	Attorney for Applicants

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary  
Amendment prior to examination of the above-identified application on the merits  
by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

At page 1, line 3, after "August 1, 1995.", please insert This

A1 application is also a continuation-in-part of U.S. Patent Application Serial No.

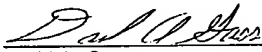
08/340,011, filed November 14, 1994.

**REMARKS**

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

Respectfully submitted,

Dated: August 12, 1996

  
David A. Gass  
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300



**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s):	)	Title: Receptor Ligand
	)	
Alitalo et al.	)	
	)	
Serial No: 08/585,895	)	Group Art Unit: Not yet assigned
	)	
Filed: January 12, 1996	)	Examiner: Not yet assigned
	)	

**TRANSMITTAL LETTER**

*Assistant Commissioner for Patents  
Washington, D.C. 20231*


Sir:

Transmitted herewith are a Preliminary Amendment and an executed Inventors' Declaration for the above application.

---

**CERTIFICATE OF MAILING (37 CFR 1.8)**

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: August 12, 1986

By: David A. Gass

David A. Gass  
Reg. No: 38,153



**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s):	)	Title: Receptor Ligand
Alitalo et al.	)	
Serial No: 08/585,895	)	Group Art Unit: Not yet assigned
Filed: January 12, 1996	)	Examiner: Not yet assigned
	)	

**TRANSMITTAL LETTER**

***Assistant Commissioner for Patents  
Washington, D.C. 20231***

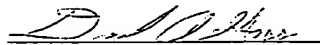
Sir:

Transmitted herewith are a Preliminary Amendment and an executed Inventors' Declaration for the above application.

---

**CERTIFICATE OF MAILING (37 CFR 1.8)**

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David A. Gass

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- ☒ Has not been established.

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Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: April 12, 1996

By: David A. Gass

David A. Gass  
Reg. No: 38,153





Atty. Docket No: 28113/33072

## DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a being named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed  
☐ Yes ☒ No

950624	Finland	13 February 1995
(Application Serial Number)	(Country)	(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

	(Day/Month/Year Filed)
(Application Serial Number)	

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

08/340,011	14 November 1994	Pending
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)
08/510,133	01 August 1995	Pending
(Application Serial Number)	(Day/Month/Year Filed)	(Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**POWER OF ATTORNEY:** I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)  
Donald J. Brott (19,490)  
Owen J. Murray (22,111)  
Allen H. Gerstein (22,218)  
Nate F. Scarpelli (22,320)  
Edward M. O'Toole (22,477)  
Michael F. Borun (25,447)

Trevor B. Joika (25,542)  
Timothy J. Vezasu (26,348)  
Carl E. Moore, Jr. (26,487)  
Richard H. Anderson (26,526)  
Patrick D. Ertel (26,877)  
James P. Zeller (28,491)  
William E. McCracken (30,195)

Richard A. Schurr (30,890)  
Anthony Nimmo (30,920)  
Christine A. Dudzik (31,245)  
Kevin D. Hogg (31,839)  
Jeffrey S. Sharp (31,879)  
Donald J. Pochopien (32,167)  
Martin J. Hirsch (32,237)

James J. Napoli (32,361)  
Richard M. La Barge (32,254)  
Jeffrey W. Smith (33,455)  
Douglas C. Hochstetler (33,710)  
Cynthia L. Schaller (34,245)  
Robert M. Gerstein (34,824)  
David A. Gass (38,153)

Send correspondence to: David A. Gass

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60606-6402

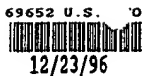
Full Name of First or Sole Inventor Kari Alitalo	Citizenship Finland
Residence Address - Street Nyyrikintie 4A	Post Office Address - Street Same
City (Zip) 02100 Espoo	City (Zip) Same
State or Country FINLAND <i>Fix</i>	State or Country Same
Date <i>Aug 6 1996</i>	Signature <i>John O'Connell</i>

☐ See second page for additional inventor

See reverse for relevant rules &amp; statutes

Second Joint Inventor, if any Vladimir Joukov	Citizenship Russia
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki	City (Zip) Same
State or Country FINLAND <i>Fin</i>	State or Country Same
Date Aug. 6, 1996	Signature V. Joukov

*ALL  
pages of #20  
are being  
changed*



#9  
1-13-97  
mu  
PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	I hereby certify that this paper and the
	)	documents referred to as enclosed
Alitalo et al.	)	herewith are being deposited with the
	)	United States Postal Service as First
Serial No.: 08/585,895	)	Class Mail, postage prepaid, in an
	)	envelope addressed to: Assistant
Filed: January 12, 1996	)	Commissioner for Patents, Washington,
	)	DC 20231, on this date:
For: "Receptor Ligand"	)	Date: <u>December 19, 1996</u>
	)	<u>David A. Gass</u>
Group Art Unit: 1801	)	David A. Gass
	)	Reg. No.: 38,153
Examiner: Lathrop, B.	)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of the listed documents are enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by

RECEIVED  
JAN 10 1997  
GROUP 1800

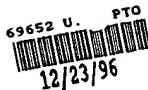
the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: December 19, 1996

By: David A. Gass  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	I hereby certify that this paper and the
Alitalo et al.	)	documents referred to as enclosed
Serial No.: 08/585,895	)	herewith are being deposited with the
Filed: January 12, 1996	)	United States Postal Service as First
For: "Receptor Ligand"	)	Class Mail, postage prepaid, in an
Group Art Unit: 1801	)	envelope addressed to: Assistant
Examiner: Lathrop, B.	)	Commissioner for Patents, Washington,
	)	DC 20231, on this date:
	)	Date: <u>December 19, 1996</u>
	)	<u>David A. Gass</u>
	)	David A. Gass
	)	Reg. No.: 38,153
	)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

RECEIVED  
JAN 10 1997  
GROUP 1800

Assistant Commissioner for Patents  
Washington, D.C. 20231

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the Patent Office without payment of a fee. See 37 C.F.R. 51.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: December 19, 1996

By: David A. Gass

David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



#613  
I.D.S. 4/29/97  
PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	I hereby certify that this paper and the
Alitalo et al.	)	documents referred to as enclosed
Serial No.: 08/585,895	)	herewith are being deposited with the
Filed: January 12, 1996	)	United States Postal Service as First
For: "Receptor Ligand"	)	Class Mail, postage prepaid, in an
Group Art Unit: 1801	)	envelope addressed to: Assistant
Examiner: Lathrop, B.	)	Commissioner for Patents, Washington,
	)	DC 20231, on this date:
	)	Date: <u>21 January 1997</u>
	)	<u>David A. Gass</u>
	)	David A. Gass
	)	Reg. No.: 38,153
	)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: 21 Jan. 1997

By: David A. Gass

David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300





PATENT  
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	I hereby certify that this paper and the
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Alitalo et al.	)	herewith are being deposited with the
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Serial No.: 08/585,895	)	Class Mail, postage prepaid, in an
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Filed: January 12, 1996	)	Commissioner for Patents, Washington,
	)	DC 20231, on this date:
For: "Receptor Ligand"	)	
	)	Date: <u>21 January 1997</u>
Group Art Unit: 1801	)	<u>David A. Gass</u>
	)	David A. Gass
Examiner: Lathrop, B.	)	Reg. No.: 38,153
	)	Attorney for Applicant

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: 21 Jan. 1997

By: David A. Gass  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is  
being deposited with the United  
States Postal Service as first class  
mail, postage prepaid, in an  
envelope addressed to: Assistant  
Commissioner for Patents,  
Washington, D.C. 20231, on this  
date:

Dated: 24 Jan. 1997

*David A. Gass*  
David A. Gass

AMENDMENT AND ELECTION IN RESPONSE TO RESTRICTION REQUIREMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

In an official communication dated November 25, 1996, the U.S. Patent and Trademark Office issued a restriction requirement in the above-identified patent application, and set a 30 day period for response. This amendment and election in response to the restriction requirement has been timely filed with a petition for one month extension of time and petition fee, extending the time for response until January 25, 1997.

### AMENDMENTS

#### In the claims:

Please cancel claims 2, 8-10, 12, and 14-16 without prejudice;  
amend claims 1, 3, 5, 11, and 13; and add new claims 17-25 to the application  
as shown below.

B1 sub  
C4

1. (Amended) A purified and isolated polynucleotide encoding a  
polypeptide which specifically binds to the Flt4 receptor tyrosine kinase.

B2  
sub  
C5

3. (Amended) A purified and isolated nucleic acid encoding a  
polypeptide having the amino acid sequence shown in SEQ ID NO: 33. [the  
peptide according to claim 2.]

B3 sub  
C3

5. (Amended) A vector comprising the nucleic acid according to  
claim 3 [4].

B4 sub  
C7

11. (Amended) A purified and isolated nucleic acid according to  
claim 19 wherein said polypeptide comprises approximately amino acids 1 to  
120 of SEQ ID NO: 33. [encoding the fragment of claim 10.]

B5

13. (Amended) A purified and isolated nucleic acid according to  
claim 19 wherein said polypeptide comprises approximately amino acids 1 to  
180 of SEQ ID NO: 33. [encoding the fragment of claim 12.]

B6

-17. A host cell transformed or transfected with a vector  
according to claim 5.

sub  
C8

18. A purified and isolated nucleic acid comprising a nucleotide  
sequence that encodes a polypeptide capable of binding to an Flt4 receptor  
tyrosine kinase, said polypeptide having an amino acid sequence comprising a  
portion of the amino acid sequence shown in SEQ ID NO: 33, said portion  
encoding a polypeptide capable of binding to an Flt4 receptor tyrosine kinase.

Sub  
D9  
B6  
sub  
C9

19. A purified and isolated ~~nucleic acid according to claim 18~~  
wherein said polypeptide is capable of stimulating tyrosine phosphorylation of  
Flt4 receptor tyrosine kinase.

20. A purified and isolated nucleic acid according to claim 19  
wherein said polypeptide has an apparent molecular weight of about 23 kd as  
assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

21. A purified and isolated nucleic acid according to claim 19  
wherein said polypeptide comprises an amino-terminal amino acid sequence set  
forth in SEQ ID NO: 13.

22. A purified and isolated nucleic acid according to claim 21  
wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18  
wherein said polypeptide has an apparent molecular weight of about 32 kDa as  
assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according  
to claim 24.--

**REMARKS**

This is the Applicants' second amendment to this application. A preliminary amendment, to add a priority claim to the application, was mailed on August 12, 1996.

The Applicants do not intend by the foregoing amendments or any other amendments to abandon the subject matter of any claim as originally filed or later amended, and reserve the right to claim such subject matter in other

applications, such as continuations, continuations-in-part, and divisional applications.

**I. The Applicants Elect Claims 3-7, 11, and 13 (Group II) without traverse.**

In response to the restriction requirement, the Applicants hereby elect Group II (Claims 3-7, 11, and 13), drawn to nucleic acids, vectors, and host cells.

**II. Explanation of amendments.**

Claim 1 has been amended to recite a nucleic acid, rather than a polypeptide, thereby bringing claim 1 within the scope of the elected invention of Group II.

Claim 3, which formerly depended from non-elected claim 2, has been amended to be an independent claim. Claims 11 and 13 have been amended similarly.

New claims 18 and 19 find support in Example 4 (pp. 19-21), for example.

New claim 20 finds support in Example 5 (p. 22, lines 33-34), for example.

New claim 21 finds support in Example 5 at p. 23, lines 9-10, for example.

New claim 22 finds support at p. 23, lines 9-10, and p. 30, lines 14-17, for example.

New claim 23 finds support in Example 13 (p. 31, lines 33-34), for example.

All of the pending claims (as amended herein) are properly classified with the Group II claims elected by the Applicants in response to the restriction requirement. None of the new claims introduce new matter.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: 24 Jan. 1997

By: *David A. Gass*  
David A. Gass  
Reg. No: 38,153



#. 14  
E. J. A. T. C. M. E.  
4/29/97  
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	)	Title: RECEPTOR LIGAND
	)	
Alitalo et al.	)	
	)	
Serial No: 08/585,895	)	Group Art Unit: 1801
	)	
Filed: January 12, 1996	)	Examiner: Lathrop, B.
	)	

AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents  
Washington, D.C. 20231


Sir:

Transmitted herewith is an amendment for the above application.

016 1 08585895 00210 976100 070204 115 119.00

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on January 24, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass



1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month	x	\$110.00		\$55.00
Two Months		\$390.00		\$195.00
Three Months		\$930.00		\$465.00
Four Months		\$1,470.00		\$735.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$110.00

- ☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid therefor of \$\_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$\_\_\_\_\_

Extension Fee Due With This Request \$110.00

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d)] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	17	MINUS	20	= 0	X11 =	\$	X22 =	\$
INDEP.	3	MINUS	3	= 0	X40 =	\$	X80 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+ 130 =	\$	+ 260 =	\$
TOTAL ADDITIONAL FEE						\$	OR	100.00

4. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: **\$110.00**
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: 24 Jan. 1997

By: David A. Gass  
David A. Gass  
Reg. No: 38,153



1800  
**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s):	)	Title: RECEPTOR LIGAND
Alitalo et al.	)	
Serial No: 08/585,895	)	Group Art Unit: 1801
Filed: January 12, 1996	)	Examiner: Lathrop, B.

**AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME**


*Assistant Commissioner for Patents  
Washington, D.C. 20231*

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David A. Gass

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Respectfully submitted,

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MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: 24 Jan. 1997

By: David A. Gass  
David A. Gass  
Reg. No: 38,153





Patent Application  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

The Application of: Alitalo, Kari  
and Joukov, Vladimir

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

"EXPRESS MAIL"  
Mailing label No. EMO99898621US  
Date of Deposit: February 11, 1997  
I hereby certify that this paper and the  
documents referred to as enclosed herewith are  
being deposited with the United States Postal  
Service "EXPRESS MAIL POST OFFICE TO  
ADDRESSEE" service under 37 CFR §1.10 on  
the date indicated above and is addressed to:  
Assistant Commissioner for Patents,  
Washington, D.C. 20231  
*Mark Bonadonna*  
Mark Bonadonna

INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

By: *David A. Gass*  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Feb 11, 1997

RECEIVED  
MAR 21 1997  
UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION  
23967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, RUDOLPH  
and Joukov, Vladimir

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.



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Mailing label No. EMO99898621US

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*Mark Boudonna*  
Mark Boudonna

INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

RECEIVED  
MAR 21 1997  
GROUP 12

Sir:

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

*Feb 11*, 1997

By: *David A. Gass*  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



Form PTO-1449 (Modified)		U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)			Applicant Alitalo and Joukov	
			Filing Date 01/12/96	Group 1801

## U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

## FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No

## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

<i>lan</i>	C111	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD), Development, Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22).
	C112	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors B and C and Receptors Involved in Angiogenesis," <i>German-American Academic Council Foundation (GAAC)/ Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease</i> , November 17-20, 1996, Ringberg Castle, Germany, p. 1 (ABSTRACT).
	C113	Kukk <i>et al.</i> , "VEGF-C Receptor Binding and Pattern of Expression with VEGFR-2 Suggests a Role in Lymphatic Vascular Development," <i>Development</i> , 122:3829-3837 (1996).
	C114	Paavonen <i>et al.</i> , "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," <i>IX International Vascular Biology Meeting</i> , Seattle, Washington, September 4-8, 1996, p. 76 (ABSTRACT 299).

EXAMINER <i>Enina Lathrop</i>	DATE CONSIDERED <i>5/2/97</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

SHEET 1

Form PTO-1449 (Modified)		U.S. Department of Commerce Patent and Trademark Office	App. Docket No. 28967/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)			Applicant Alitalo and Joukov	
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Gp 1801  
PATENT APPLICATION 18  
28967/33072

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:  
Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is being  
) deposited with the United States Postal Service  
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) envelope addressed to: Assistant Commissioner  
) for Patents, Washington, D.C. 20231, on this  
) date:

) Dated: MAR 21, 1997

) David A. Gass  
) David A. Gass  
) Registration No. 38,153  
) Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

RECEIVE  
APR 10 1997  
GROUP ID 1801

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed document, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of the document is enclosed herewith.

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: MAR 21, 1997

By: David A. Gass  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



PATENT APPLICATION  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:  
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Registration No. 38,153  
Attorney for Applicant

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APR 10 1997  
GROUP 1800

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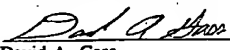
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By:

  
David A. Gass  
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Form PTO-1449 (Modified)

U.S. Department of Commerce  
Patent and Trademark OfficeApp. Docket No.  
28967/33072Serial No.  
08/585,895

## INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

Applicant  
Alitalo and JoukovFiling Date  
01/12/96Group  
1801

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611	C115	Pajusola, "Cloning and Characterization of a New Endothelial Receptor Tyrosine Kinase Flt4 and Two Novel VEGF-Like Growth Factors VEGF-B and VEGF-C," Academic Dissertation, Molecular/Cancer Biology Laboratory and Department of Pathology, Haartman Institute and Department of Biosciences, Division of Genetics, University of Helsinki, (January 26, 1996)				

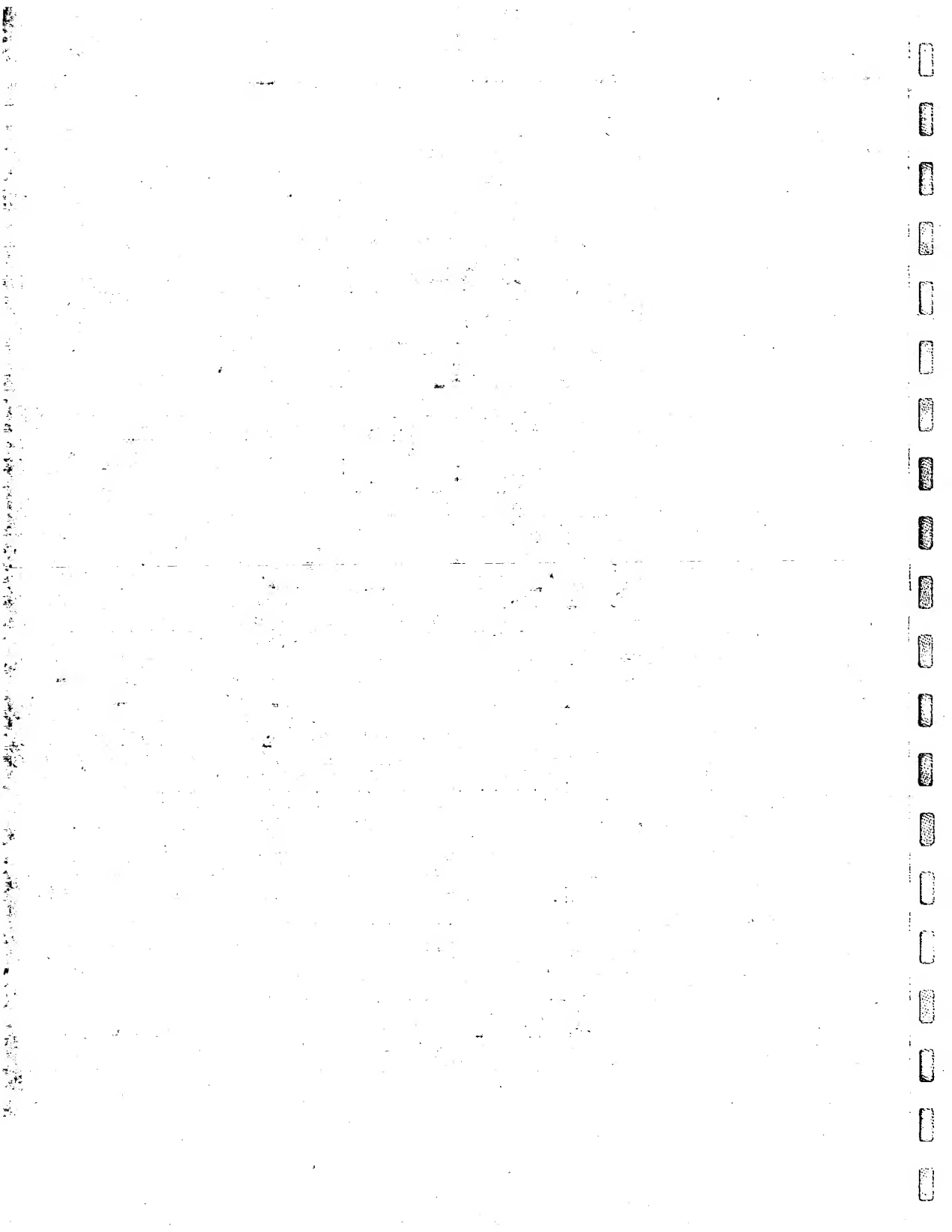
EXAMINER

Brian Lathrop

DATE CONSIDERED

5/5/97

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT  
28967/33072

Gp 1801  
#11  
5/17

In the Application of:  
Alitalo et al.  
  
Serial No.: 08/585,895  
  
Filed: January 12, 1996  
  
For: "Receptor Ligand"  
  
Group Art Unit: 1801  
  
Examiner: Lathrop, B.

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20231, on this date:  
Dated: Apr 11 1997  
  
David A. Gass  
Registration No. 38,153  
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
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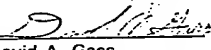
Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized on the attached form PTO-1449 was cited in a

communication (an ISR) from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

By:   
David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: Apr 16, 1997





PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: )  
Alitalo et al. )

Serial No.: 08/585,895 )

Filed: January 12, 1996 )


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Examiner: Lathrop, B. )

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20231, on this date:

Dated: April 16, 1997

  
David A. Gass  
Registration No. 38,153  
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
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6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: April 16, 1997



SHEET 1 of

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo et al.	
		Filing Date 01/12/96	Group 1801

## U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing D If Appropri

## FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
GM	B7	WO 95/33772	12/14/95	PCT	1A0		

## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

GM	C116	Hillier et al., "The WashU-Merck EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995.

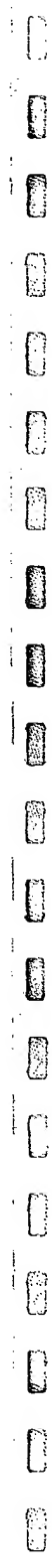
EXAMINER

Grimm Lathrop

DATE CONSIDERED

5/11/97

\*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.





**UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILED DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
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EXAMINER
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ART UNIT	PAPER NUMBER
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17

DATE MAILED:

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

**OFFICE ACTION SUMMARY**

☒ Responsive to communication(s) filed on 1/27/97 (Election/Amendment)  
☐ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

**Disposition of Claims**

☒ Claim(s) 1, 3-7, 11, 13, 17-25 is/are pending in the application.

Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.

☐ Claim(s) \_\_\_\_\_ is/are allowed.

☒ Claim(s) 1, 3-7, 11, 13, 17-25 is/are rejected.

☐ Claim(s) \_\_\_\_\_ is/are objected to.

☐ Claims \_\_\_\_\_ are subject to restriction or election requirement.

**Application Papers**

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.

☒ The specification is objected to by the Examiner.

☒ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been received.

☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

**Attachment(s)**

☒ Notice of Reference Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 8, 9, 11-13, 14

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

☒ Notice to Comply

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Application No.: **08/585895**; attachment to Paper No. **08/585895**  
**NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING  
NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES**

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

- ☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.
- ☐ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).
- ☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).
- ☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked-up "Raw Sequence Listing."
- ☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).
- ☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).
- ☒ 7. Other: Figure 10 contains sequences requiring a SEQ ID NO, and Figure 9B requires identification of protein and polynucleotide sequences by SEQ ID NO: 32 and 33. SEQ ID NOS may be added to the Brief Description of the Drawings or the Figures.

**Applicant Must Provide:**

- ☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".
- ☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.
- ☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216

For CRF Submission Help, call (703) 308-4212

For PatentIn software help, call (703) 308-6856

**PLEASE RETURN A COPY OF THIS NOTICE WITH YOUR RESPONSE**

Serial Number: 08/585895  
Art Unit: 1801

-2-

### DETAILED ACTION

#### *Election/Restriction*

1. Applicant's election without traverse of Group II, claims 3-7, 11, and 13, and amendment  
5 of claim 1 to read on the elected invention in Paper No. 15 is acknowledged.

#### *Oath/Declaration*

2. The oath or declaration is defective. A new oath or declaration in compliance with 37  
CFR 1.67(a) identifying this application by application number and filing date is required. See  
10 MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Non-initialed alterations have been made to the oath or declaration. See 37 CFR 1.52(c)  
and 1.57).

15 It does not state that the person making the oath or declaration in a continuation-in-part  
application filed under the conditions specified in 35 U.S.C. 120 which discloses and  
claims subject matter in addition to that disclosed in the prior copending application,  
acknowledges the duty to disclose to the Office all information known to the person to be  
20 material to patentability as defined in 37 CFR 1.56 which occurred between the filing date  
of the prior application and the national or PCT international filing date of the  
continuation-in-part application.

(e) the deposit will be replaced should it become necessary due to inviability, contamination, or loss of capability to function described in the manner in the specification.

5 In either case, the identifying information set forth in 37 C.F.R. 1.809(d) should be added to the specification if it is not already present. See 37 C.F.R. 1.803-1.809 for additional explanation of these requirements.

8. Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for  
10 polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for polynucleotides all polypeptides that bind the Flt4-receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

15 The scope of claim 1 encompasses polynucleotides from any source that encode polypeptides that bind specifically to the Flt4 receptor. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the encoded proteins are recited in the instant claims. There is no guidance provided by the specification to select those encompassed polynucleotides that encode proteins that specifically bind the Flt4  
20 receptor with the exception of those teachings which support the subject matter indicated as enabled. There is no guidance to predict *a priori* whether any protein would bind the receptor without some information on the structure of the protein, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention. There is no guidance provided by the state of the art to select ligands to make the invention; despite intense



Serial Number: 08/585895  
Art Unit: 1801

-6-

research in this area, Borg et al. (reference C7) teach that no known ligands for the Flt4 receptor were known at the time of the invention. The amount of guidance required varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). See also *Genentech, Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

9. Claims 18-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for the scope of polynucleotides commensurate with the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The breadth of claims 18-25 encompasses polynucleotides encoding all active fragments of the protein of SEQ ID NO:33, or those that are 23 kDa or 32 kDa in size. Claim 21 recites the limitation that the fragment comprises SEQ ID NO:13, but this claim encompasses polypeptides in which SEQ ID NO:13 is the only sequence derived from the protein of SEQ ID NO:33. The only

guidance offered by the specification to those fragments that may make the invention is provided at page 11 where at least residues 1-120 of SEQ ID NO:33 are taught to be required for activity by comparison to PDGF (*infra*). Heldin et al. (reference C40), however, teach the criticality of structures throughout the corresponding region of PDGF, such as extended loop structures (Figure 2), disulfide bonds involving residue 1 (page 249, column 1), and specific residues including those up to residue 154 (*loc. cit.*). Moreover, comparison with PDGF is of limited predictive value, because the stereo-specific interaction required between VEGF-C and its receptor are different than those between PDGF and its receptor as evidenced by the fact that the two ligands do not bind the same receptors. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Recitation that the encoded proteins must be 23 or 32 kDa in size does not provide significant additional guidance or limitation to the scope of the claims, because this limitation does not exclude the presence of sequences unrelated to VEGF-C within the polypeptide, nor does it exclude various post-translational modifications known to profoundly influence the apparent molecular weight without affecting the primary sequence of the polypeptide. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation required to test all the encompassed fragments is an additional factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth, undue experimentation would be required to make

08/1

Serial Number: 08/585895  
Art Unit: 1801

-8-

the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

10. Claims 3-5, 11, 13, and 17-25 are rejected under 35 U.S.C. 112, second paragraph, as  
5 being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

SEQ ID NO:32 encodes a protein whose amino terminus is indicated as residue 1, and the specification teaches that the amino terminal of a 23 kDa protein expressed from the polynucleotide has the amino terminus shown in SEQ ID NO:13; however, Human Genome  
10 Sciences, Inc, disclose DNA encoding a similar sequence (99% global identity using the Smith-Waterman algorithm with 100% identity to the instantly claimed protein in the amino terminus through the instant residue 8) whose amino terminus is indicated as residue -8 of the instant protein. It would have been understood in the art that a disclosure of a particular residue as  
"residue 1" would have meant that this residue was the amino terminus of the mature polypeptide;  
15 however, it was unclear which residue corresponds to the amino terminus of the encoded polypeptide, making the designation of a particular amino acid residue as a "residue 1" indefinite. Although Human Genome Sciences was published after the effective filing date, the publication is used to show that the instant claims were indefinite at the time of filing. MPEP 2124 citing *In re Glass*, 492 F.2d 1228, 1232 n.6., 181 USPQ 31, 34 n.6 (CCPA 1974).

Serial Number: 08/585895  
Art Unit: 1801

-9-

11. Claims 11 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the term "approximately" in claims 11 and 13 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the  
5 specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

*Allowable Subject Matter*

12. Claims 3-5, 11, 13, and 17 would be allowable over the prior art of record if rewritten or  
10 amended to overcome the rejection(s) under 35 U.S.C. 112 set forth in this Office action.

13. The following is a statement of reasons for the indication of allowable subject matter: polynucleotides encoding the instantly claimed ligand appear to be novel over the prior art of record. Borg et al. (reference C7), for example, disclose that the ligand for Flt4 was not known in  
15 the art around the time of invention. Closest prior art is a DNA with about 99% identity to the claimed polynucleotide (Human Genome Sciences, Inc., reference B1), but the publication date antedates the effective filing date of the instant application. Other relevant prior art made of record below discloses a series of expressed sequence tags (ESTs) with high identity to large regions of the Flt4 ligand cDNA. The probable identity of these ESTs was not disclosed, and  
20 without the benefit of hindsight, the artisan at the time of invention would not have been motivated to use these ESTs to make the claimed invention. It was not known that these ESTs

Serial Number: 08/585895  
Art Unit: 1801

-10-

encoded a receptor ligand, nor was it known that these ESTs were nearly identical to the Flt4 ligand cDNA at the time of invention. In fact, the only EST posited to encode a particular protein was taught to encode a Balbiani ring protein (Hillier et al., EST-ST5 Accession No. T81690), hardly giving motivation to use the EST to find the claimed invention.

5

#### *Conclusion*

14. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

- Eriksson et al. disclose VEGF-B and DNA encoding this protein, which appears to be structurally distinct from the instantly claimed DNAs. It does not appear that the DNAs disclosed by Eriksson et al. encode proteins that would reasonably have the inherent property of meeting the claim limitation of binding the Flt4 receptor.

15. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Stephen Walsh, can be reached at (703) 308-2957. The FAX number for Art Unit 1801 is (703) 305-7401.

20. An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.

*BKL*  
Brian K. Lathrop, Ph.D.  
5/25/97

  
DAVID L. FITZGERALD  
PRIMARY EXAMINER  
GROUP 1800

## NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or informal. Additionally, patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch, 703-305-8404.

The drawings filed (insert date) 1/12/06A. not objected to by the Draftsperson under 37 CFR 1.84 or 1.152.

B. not objected to by the Draftsperson under 37 CFR 1.84 or 1.152 as indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawings must be submitted according to the instructions on the back of this Notice.

## 1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:

- Black ink. Color.  
 Not black solid lines. Fig(s) \_\_\_\_\_  
 Color drawings are not acceptable until petition is granted.  
 Fig(s) \_\_\_\_\_

## 2. PHOTOGRAPHS. 37 CFR 1.84(b)

- Photographs are not acceptable until petition is granted.  
 Fig(s) \_\_\_\_\_  
 Photographs not properly mounted (must use bristol board or photographic double-weight paper). Fig(s) \_\_\_\_\_  
 Poor quality (half-tone). Fig(s) \_\_\_\_\_

## 3. GRAPHIC FORMS. 37 CFR 1.84(d)

- Chemical or mathematical formula not labeled as separate figure.  
 Fig(s) \_\_\_\_\_  
 Group of waveforms not presented as a single figure, using common vertical axis with time extending along horizontal axis.  
 Fig(s) \_\_\_\_\_  
 Individuals waveform not identified with a separate letter designation adjacent to the vertical axis. Fig(s) \_\_\_\_\_

## 4. TYPE OF PAPER. 37 CFR 1.84(c)

- Paper not flexible, strong, white, smooth, nonshiny, and durable.  
 Sheet(s) \_\_\_\_\_  
 Erasures, alterations, overwritings, interlineations, cracks, creases, and folds copy machine marks not accepted. Fig(s) \_\_\_\_\_  
 Mylar, velum paper is not acceptable (too thin). Fig(s) \_\_\_\_\_

## 5. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:

- 21.6 cm. by 35.6 cm. (8 1/2 by 14 inches)  
 21.6 cm. by 33.1 cm. (8 1/2 by 13 inches)  
 21.6 cm. by 27.9 cm. (8 1/2 by 11 inches)  
 21.0 cm. by 29.7 cm. (DIN size A4)  
 All drawing sheets not the same size. Sheet(s) \_\_\_\_\_  
 Drawing sheet not an acceptable size. Sheet(s) \_\_\_\_\_

## 6. MARGINS. 37 CFR 1.84(g): Acceptable margins:

## Paper size

21.6 cm. X 35.6 cm. (8 1/2 X 14 inches)	21.6 cm. X 33.1 cm. (8 1/2 X 13 inches)	21.6 cm. X 27.9 cm. (8 1/2 X 11 inches)	21.0 cm. X 29.7 cm. (DIN Size A4)
T 51 cm. (20")	25 cm. (10")	25 cm. (10")	25 cm. (10")
L 64 cm. (25")	64 cm. (25")	64 cm. (25")	25 cm. (10")
R 64 cm. (25")	64 cm. (25")	64 cm. (25")	15 cm. (6")
B 64 cm. (25")	64 cm. (25")	64 cm. (25")	15 cm. (6")

Margins do not conform to sheet above.

- Sheet(s) \_\_\_\_\_  
 Top (T) \_\_\_\_\_ Left (L) \_\_\_\_\_ Right (R) \_\_\_\_\_ Bottom (B) \_\_\_\_\_

## 7. VIEWS. 37 CFR 1.84(h)

- REMINDER: Specification may require revision to correspond to drawing changes.  
 All views not grouped together. Fig(s) \_\_\_\_\_  
 Views connected by projection lines or lead lines.  
 Fig(s) \_\_\_\_\_  
 Partial views. 37 CFR 1.84(h) 2

View and pattern view not titled separately or properly. Fig(s) \_\_\_\_\_

Sectional views. 37 CFR 1.84 (h) 3

Hatching not indicated for sectional portions of an object. Fig(s) \_\_\_\_\_

Cross section not drawn same as view with parts in cross section with regularly spaced parallel oblique strokes. Fig(s) \_\_\_\_\_

## 8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)

- Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) \_\_\_\_\_

## 9. SCALE. 37 CFR 1.84(j)

- Scale not large enough to show mechanism with crowding when drawing is reduced in size to two-thirds in reproduction.  
 Fig(s) \_\_\_\_\_  
 Indication such as "actual size" or scale 1/2" not permitted.  
 Fig(s) \_\_\_\_\_

## 10. CHARACTER OF LINES, NUMBERS, &amp; LETTERS. 37 CFR 1.84(k)

- Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (except for color drawings).  
 Fig(s) \_\_\_\_\_

## 11. SHADING. 37 CFR 1.84(m)

- Solid black shading areas not permitted.  
 Fig(s) \_\_\_\_\_  
 Shade lines, pale, rough and blurred. Fig(s) \_\_\_\_\_

## 12. NUMBERS, LETTERS, &amp; REFERENCE CHARACTERS. 37 CFR 1.84(p)

- Numbers and reference characters not plain and legible. 37 CFR 1.84(p)(1) Fig(s) \_\_\_\_\_  
 Numbers and reference characters not oriented in same direction as the view. 37 CFR 1.84(p)(1) Fig(s) \_\_\_\_\_  
 English alphabet not used. 37 CFR 1.84(p)(2) Fig(s) \_\_\_\_\_  
 Numbers, letters, and reference characters do not measure at least 32 of 36 inches high. 37 CFR(p)(3) Fig(s) \_\_\_\_\_

## 13. LEAD LINES. 37 CFR 1.84(q)

- Lead lines cross each other. Fig(s) \_\_\_\_\_  
 Lead lines missing. Fig(s) \_\_\_\_\_

## 14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(o)

- Sheets not numbered consecutively, and in Arabic numerals, beginning with number 1. Sheet(s) \_\_\_\_\_

## 15. NUMBER OF VIEWS. 37 CFR 1.84(u)

- Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) \_\_\_\_\_  
 View numbers not preceded by the abbreviation Fig. Fig(s) \_\_\_\_\_

## 16. CORRECTIONS. 37 CFR 1.84(w)

- Corrections not made from prior PTO-948.  
 Fig(s) \_\_\_\_\_

## 17. DESIGN DRAWING. 37 CFR 1.152

- Surface shading shown not appropriate. Fig(s) \_\_\_\_\_  
 Solid black shading not used for color contrast.  
 Fig(s) \_\_\_\_\_

## COMMENTS:

Fig. legend placed incorrectly (Fig. 9A, 15B, 16A)

1.1AC

9/17/06

<b>Notice of References Cited</b>				Application No. 08/585,895		Applicant(s) Alitalo et al.	
				Examiner Brien Lathrop		Group Art Unit 1801	
Page 1 of 1							
<b>U.S. PATENT DOCUMENTS</b>							
		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	
A		5,607,918	3/4/97	Eriksson et al.	514	12	
B							
C							
D							
E							
F							
G							
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<b>FOREIGN PATENT DOCUMENTS</b>							
		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
N							
O							
P							
Q							
R							
S							
T							
<b>NON-PATENT DOCUMENTS</b>							
		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)					DATE
	U	Hillier et al. y185b08.21 Homo sapiens cDNA clone 44993 5'. EST-STS Accession No. H05177.					6/21/95
	V	Hillier et al. y186g06.r1 Homo sapiens cDNA clone 45138 5'. EST-STS Accession No. H07991.					6/23/95
	W	Hillier et al. yd29107.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE 003376 BALBIANI RING PROTEIN 3. EST-STS Accession No. T81690.					3/15/95
	X	Auffray et al. H. sapiens partial cDNA sequence; clone c-1wf11. EST-STS Accession No. Z44272.					11/6/94

*Brien Lathrop 5/25/97*

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/33072	Serial No. 08/585,895
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary) 15		Applicant Alitalo and Joukov	
		Filing Date 01/12/96	Group 1806 1801

## U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
BUL	A1	5,332,671	07/26/94	Ferrara <i>et al.</i>	435	240.1
BUL	A2	5,219,739	06/15/93	Tischer <i>et al.</i>	435	69.4

## FOREIGN PATENT DOCUMENTS

*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation	
						Yes	No
BUL	B1	WO 95/24473 A1	09/14/95	PCTWO	—	—	
BUL	B2	WO 96/11269 A2	04/18/96	PCTWO	—	—	

## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

BUL	C1	Andersson <i>et al.</i> , "Assignment of Interchain Disulfide Bonds in platelet-Derived Growth Factor (PDGF) and Evidence for Agonist Activity of Monomeric PDGF," <i>J. Biol. Chem.</i> , 267(16):11260-11266 (June 5, 1992).				
	C2	Aprelikova <i>et al.</i> , "FLT4, A Novel Class III Receptor Tyrosine Kinase in Chromosome 5q33-qter," <i>Cancer Research</i> , 52:746-748 (February 1, 1992).				
	C3	Ausprunk, <i>et al.</i> , "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis," <i>Microvasc. Res.</i> , 14:53-65 (1977).				
	C4	Basilico <i>et al.</i> , "The FGF Family of Growth Factors and Oncogenes," <i>Adv. Cancer Res.</i> , 59:145-165 (1992).				
	C5	Berse <i>et al.</i> , "Vascular Permeability Factor (Vascular Endothelial Growth Factor) Gene is Expressed Differentially in Normal Tissues, Macrophages, and Tumors," <i>Mol. Biol. Cell.</i> , 3:211-220 (February, 1992).				
	C6	Betsholtz <i>et al.</i> , "cDNA Sequence and Chromosomal Localization of Human Platelet-Derived Growth Factor A-Chain and Its Expression in Tumor Cell Lines," <i>Nature</i> , 320:695-699 (April, 1986).				

EXAMINER Erica Lathrop	DATE CONSIDERED 5/8/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



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<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo et al.	
		Filing Date 01/12/96	Class 1806 / 821

## U.S. PATENT DOCUMENTS

*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing I If Appropriat

## FOREIGN PATENT DOCUMENTS

*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
BLL	B5	WO 96/39421	12/12/96	PCT-WO	—	—		
BLL	B6	WO 96/39515	12/12/96	PCT-WO	—	—		

## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)


EXAMINER Erin Lathrop	DATE CONSIDERED 5/8/97
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#11  
01/16/97

**PATENT**  
**28967/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:	)	I hereby certify that this paper is
Alitalo et al.	)	being deposited with the United
Serial No. 08/585,895	)	States Postal Service as first class
Filed: January 12, 1996	)	mail, postage prepaid, in an
For: RECEPTOR LIGAND	)	envelope addressed to: Assistant
Art Unit: 1801	)	Commissioner for Patents,
Examiner: Lathrop, B.	)	Washington, D.C. 20231, on this
	)	date:
	)	Dated: <u>November 26, 1997</u>
	)	<u>David A. Gass</u>
	)	David A. Gass

**AMENDMENT AND REPLY PURSUANT TO**  
**37 C.F.R. §§ 1.111 AND 1.115**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

In an official action mailed May 28, 1997, the U.S. Patent and Trademark Office (the Patent Office) rejected claims 1, 3-7, 11, 13, and 17-25 variously under 35 U.S.C. §§ 112, first and second paragraphs. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This Amendment and Reply has been timely filed with a petition and fee for three months extension of time, extending the time period for response until November 28, 1997.

## AMENDMENTS

### In the specification:

Please amend the specification as follows:

At page 4, lines 14 and 21, delete "Flt-4" and substitute therefor --

Flt4 --

At page 5, line 14, delete "32" and substitute -- 33 --.

At page 5, line 17, delete "318" and substitute -- 317 --.

At page 6, line 31, delete "97321" and substitute therefor --

97231 --.

At page 8, line 14, delete "Figure 5 shows" and substitute therefor -- Figures 5A, 5B, and 5C show --.

At page 8, lines 19-20, delete "fractions from the Western analysis" and substitute therefor -- chromatographic fractions from the affinity purification --.

At page 8, please delete the brief description of Figure 10 at lines 30-32, and substitute therefor:

Figures 10A-10E show a comparison of the deduced amino acid sequences of PDGF-A (SEQ ID NO: 36); PDGF-B (SEQ ID NO: 37); two PIGF isoforms (SEQ ID NOs: 38 and 39); four VEGF isoforms (SEQ ID NOs: 40-43); and Flt4 ligand (VEGF-C) (SEQ ID NO: 33).---

At page 9, line 5, after "lines" please insert -- and in brain tissue --.

At page 9, lines 6 and 10, delete "VEFG-C" and substitute therefor -- VEGF-C --.

At page 9, line 27, delete "its cloning" and substitute therefor -- the cloning of a DNA encoding this growth factor --.

At page 10, lines 1-2, delete "Claimed ligands" and substitute therefor -- Ligands of the invention --.

At page 11, line 7, after "residues" insert -- of --.

At page 11, line 19, delete "Balbaini" and substitute therefor -- Balbiani --.

At page 12, line 2, delete "have" and substitute therefor -- has --.

At page 12, line 7, delete "to structure to" and substitute therefor -- in structure to --.

At page 12, line 23, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 12, line 25, after "cells" insert -- (BCE) --.

At page 13, line 14, delete "the".

At page 13, line 17, delete "diseases" and substitute therefor -- diseases --.

At page 13, line 17, delete "to".

At page 14, line 4, delete "genes" and substitute therefor -- gene --.

At page 14, line 15, delete "these genes" and substitute therefor -- this gene --.

At page 14, line 20, delete "have" and substitute therefor -- has --.

At page 15, line 6, delete "the".

At page 15, line 12, after "Centricon 100" insert -- filters --.

At page 17, line 3, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 17, lines 9 and 13, delete "analysed" and, in each instance, substitute therefor -- analyzed --.

At page 17, line 13, after "50  $\mu$ g" insert -- of --.

At page 17, line 13, delete "was" and substitute therefor -- were --.

At page 17, line 18, delete "carboxyterminal" and substitute therefor -- carboxy-terminal --.

At page 19, lines 26 and 28, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 20, line 19, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 20, line 30, delete "was" and substitute therefor -- were --

At page 21, line 16, delete "3" and substitute therefor -- 4 --

At page 21, line 27, delete "extracellular" and substitute therefor -- extracellular --

At page 22, line 5, delete "dialysed" and substitute therefor -- dialyzed --

At page 22, line 14, delete "NIH3T3" and substitute therefor -- NIH 3T3 --

At page 23, line 4, delete "Malborough" and substitute therefor -- Marlborough --

At page 23, line 19, delete "was" and substitute therefor -- were --

At page 24, line 21, delete "analysed" and substitute therefor -- analyzed --

At page 25, line 29, delete "analysed" and substitute therefor -- analyzed --

At page 27, line 17, delete "analysed" and substitute therefor -- analyzed --

At page 27, line 33, delete "32" and substitute therefor -- 33 --

At page 28, line 17, delete "NIH3T3" and substitute therefor -- NIH 3T3 --

At page 28, line 19, delete "analysed" and substitute therefor -- analyzed --

At page 28, line 26, delete "slur" and substitute therefor -- slurry --

At page 29, line 1, after "97231.", please insert -- A 1997 base

pair nucleotide sequence of the cDNA insert of this deposited plasmid is set forth in SEQ ID NOs: 44 and 45. --

At page 29, line 11, delete "two" and substitute therefor -- three --

At page 29, line 17, delete "Balbiani ring protein 3 (BRP3)" and substitute therefor -- Balbiani ring 3 protein (BR3P) --.

At page 29, line 22, delete "BRP3" and substitute therefor -- BR3P --.

At page 30, line 9, delete "assayed" and substitute therefor -- assayed --.

At page 30, line 11, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 30, line 34, delete "be" --.

At page 31, line 4, delete "analysed" and substitute therefor -- analyzed --.

At page 31, line 7, delete "10<sup>9</sup>" and substitute therefor -- 10<sup>8</sup> --.

At page 31, line 23, delete "Metabolical" and substitute therefor -- metabolic --.

At page 31, line 30, delete "30 ml of a slur" and substitute therefor -- 30  $\mu$ l of a slurry --.

At page 32, line 3, delete "receptor binding" and substitute therefor -- receptor-binding --.

At page 32, line 24, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 32, line 34, delete "ml" and substitute therefor --  $\mu$ l --.

At page 33, line 3, delete "TBS" and substitute therefor -- RIPA --.

At page 33, lines 6, 14, and 25, delete "analysed" and, in each instance, substitute therefor -- analyzed --.

At page 33, line 19, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 33, line 31, after "(" please insert -- Fig. 14A --.

At page 34, line 10, delete "analysed" and substitute therefor -- analyzed --.

At page 34, line 14, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 36, line 20, delete "highly expressed in all tissues analysed" and substitute therefor -- highly expressed in all tissues analyzed --.

At page 37, line 19, delete "Gaithersburg" and substitute therefor -- Gaithersburg --.

At page 38, line 3, delete "analysed" and substitute therefor -- analyzed --.

At page 38, line 12, delete "VEGF-B" and substitute therefor -- VEGF-C --.

At page 38, line 27, delete "c6" and substitute therefor -- C6 --.

At page 39, line 16, delete "20952" and substitute therefor -- 20852 --.

Please delete pages 40-49 of the specification, which comprise the original sequence listing, and substitute therefor new pages 40-60 filed herewith, which constitute a substitute Sequence Listing. In view of this amendment, please renumber the pages of claims and abstract beginning with "61" (to preserve consecutive page numbering).

In the claims:

Please cancel claims 6, 13, and 17; amend claims 1, 3-5, 7, 11, 18, and 20; and add new claims 26-38 as shown below:

1. (Twice amended) A host cell transformed or transfected with a [purified and isolated] polynucleotide encoding a polypeptide (which specifically binds) that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and



(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

C4  
correl.  
wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

3. (Twice amended) A host cell transformed or transfected with a [purified and isolated] nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

C5  
4. (Amended) A host cell [The nucleic acid] according to claim 3 wherein said nucleic acid comprises [having] the sequence shown in SEQ ID NO: 32.

5. (Twice amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising a nucleic acid that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 33 [the nucleic acid according to claim 3].

*Sub D7  
C6*  
7. (Amended) A host cell comprising plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses a polypeptide encoded by said plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P) (the vector according to claim 6).

*C7*  
11. (Twice amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises (approximately) amino acids 1 to 120 of SEQ ID NO: 33.

*Sub D8  
C8*  
18. (Amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33[, said portion encoding a polypeptide capable of binding to an Flt4 receptor tyrosine kinase] effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

*C9*  
20. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 [kd] kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

*C10*  
26. A host cell according to claim 1 that expresses a naturally occurring VEGF-C protein encoded by said polynucleotide.

*Sub D10*  
27. A host cell according to claim 1 that expresses a human VEGF-C protein encoded by said polynucleotide.

28. A host cell according to claim 27, wherein said host cell expresses said polynucleotide and produces a mature human VEGF-C protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to a nucleotide sequence that encodes said polypeptide.

30. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes a VEGF-homologous portion of SEQ ID NO: 33 and excludes the portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

C<sup>10</sup>  
cont.  
31. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

37. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight

conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

C<sup>10</sup>  
word.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell. --

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## **REMARKS**

### **I. History of claims and explanation of amendments.**

#### **A. Prosecution History**

The application as filed contained 16 claims.

In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In the outstanding Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In the present amendment, the Applicants cancel claims 6, 13, and 17; amend claims 1, 3-5, 7, 11, 18, and 20; and add new claims 26-38. Thus, claims 1, 3-5, 7, 11 and 18-38 are now pending. A copy of the claims, in their amended forms, is appended hereto for the Examiner's convenience.

The Applicants do not intend by the amendments herein or any other amendments to abandon the subject matter of any claim as originally filed or as previously amended, and reserve the right to pursue such subject matter in subsequent applications, such as continuations, continuations-in-part, and divisional applications.

#### **B. Amendments to the specification.**

Most of the amendments to the specification correct obvious typographical errors, grammatical errors, and the like.

The amendments at page 5, lines 14 and 17, correct obvious typographical errors in the designation of the portions of SEQ ID NO: 33 which correspond to the unprocessed and "mature" forms of VEGF-C. The description of the amino terminus of a mature form of VEGF-C is found in the specification at, e.g., p. 23, lines 5-10, and is confirmed at page 25, line 27, to page 26,

line 6 (from which it is apparent that the first 13 amino acid residues of a secreted Flt4 ligand are encoded by the thirty-nine 3' bases of SEQ NO: 25 that begin ACAGAAGAGACT...). From these excerpts of the specification that identify the amino terminus of a mature VEGF-C protein, it is clear that the residues of SEQ ID NO: 33 as originally filed were misnumbered. As explained in the accompanying statement, a corrected SEQ ID NO: 33 has been filed herewith.

The amendment at page 6 to correct the ATCC accession number corrects an obvious typographical error, as is apparent from the ATCC deposit information provided at page 39, lines 14-18.

The amendment to the description of Figure 7 at page 8, lines 19-20; finds support at pages 21-22 (Example 5), from which it is apparent that Figure 7 depicts the results of gel electrophoresis of chromatographic fractions from the affinity purification of the Flt4 ligand.

The description for Figure 10 has been amended to reflect that Figure 10 is presently two panels (Figs. 10A-10B) and to include cross-references to the amended sequence listing. These amendments are responsive to objections raised in paragraphs 5 and 6 of the Office action.

The amendment at page 9, line 5, to add "brain tissue" to the description of Figure 11 finds support in Figure 11 itself, wherein the gel lane depicting the results for brain tissue is clearly labeled.

The amendments at pages 11 and 29 to correctly designate "Balbiani ring 3 protein" find support in the articles referenced at page 11, and the correct designations would have been understood by one skilled in the art.

The corrected cross-reference to Example 4 at page 21, line 16, finds support in Example 4 itself, because it is readily apparent from reading the application that Example 4 characterizes the ligand expressed by PC-3 cells.

The amendment at page 27, line 33, finds support as described above for the similar amendment at page 5, line 14.

The amendments to add SEQ ID NOs: 44-45 and include a cross-reference thereto at page 29, line 1, find support in the deposited plasmid

pFLT4-L, as an inherent property of the plasmid. See *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985); *Therma-Tru Corp v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276 (Fed. Cir. 1995) ("[T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); and *Kennecott Corp. v. Kyocera International Inc.*, 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date).

The amendment at page 29, line 11 finds support in Figure 10, wherein three (not two) putative N-linked glycosylation sites are underlined.

The substitution of " $\mu$ l" for "ml" at page 32, line 33, would have been obvious to the person of ordinary skill in the art from the context of Example 14, due to the nature of the experiment.

The corrected designation of a wash buffer used in the VEGFR-2 binding experiments at page 33, line 3 (Example 14), improves the readability of the eventual patent. This correction does not relate to how one would make or use the subject matter of the claims.

The amendment at page 33, line 31, to provide a cross-reference to Fig. 14A finds support in the context of the discussion of Figs. 14A-14B at page 33 and in the figures themselves, and will improve the readability of the eventual patent.

Support for the amendment at page 38, line 12, to substitute "VEGF-C" for "VEGF-B" is apparent from the context at page 38, lines 10-18, from which it is apparent that VEGF-C-encoding DNA is being discussed.

Support for the substitute Sequence Listing filed herewith is provided in the accompanying statement filed herewith.

**C. Amendments to the claims.**

All of the claim amendments find support throughout the application as originally filed.



For example, the recitations in claim 1 relating to binding to the extracellular domain of human Flt4 find support at p. 5, lines 4-9; p. 9, lines 30-32; p. 10, lines 26-31; p. 14, lines 30-34; and Examples 4 and 5.

The hybridization conditions recited in claim 1 find support in Example 10 (especially at p. 27, lines 9-14), wherein the recited hybridization conditions were employed in the isolation of VEGF-C-encoding cDNAs from a cDNA library.

The recitations in claims 1, 3, 7, 18, and 30 regarding expression of a polypeptide that includes a VEGF-homologous domain but excludes any domain having homology to a Balbiani ring 3 protein finds support in Example 13 (teaching that transfected host cells express and secrete 32 kD and 23 kD forms of VEGF-C that bind Flt4); at p. 11, lines 11-23 (teaching that the 23 kD polypeptide is likely to represent the VEGF-homologous domain, and that the carboxy-terminal amino acid sequences that show a cysteine pattern reminiscent of the Balbiani ring 3 protein is at least partially cleaved off); at page 11, lines 33-35, and in Fig. 10 (describing and depicting the eight conserved cysteine residues of the PDGF/PIGF/VEGF family of proteins).<sup>1</sup>

The recitation of plasmid pFLT4-L in claim 7 finds support in claim 6 (from which claim 7 originally depended).

The amendment of claim 20 to substitute "kD" for "kd" is not intended as a substantive change, but merely is intended to increase uniformity of the eventual patent.

The recitation of an expression vector in claim 29 finds support in Example 11, e.g., at p. 28, lines 5-13; and at p. 6, lines 27-31.

The amino acid ranges recited in claims 31 and 33 find support at page 5, lines 27-33.

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<sup>1</sup> In Fig. 10, the eight conserved cysteines are readily apparent at positions 103, 130, 136, 139, 140, 147, 184, and 186. In SEQ ID NO: 33 of the amended sequence listing, these eight cysteines correspond to residues 29, 54, 60, 63-64, 71, 107, and 109.

Support for claims 36-38, directed to a method for producing a polypeptide with host cells of the invention, is found at p. 6, lines 32-35, for example.

**II. The second inventors' declaration, filed August 12, 1996, is not defective.**

In paragraph 2 of the Office action, the Patent Office alleged that the inventors' declaration was defective due to non-initialed alterations and failure to acknowledge that the application is a CIP. The alleged defects are rendered moot by the second inventors' declaration that accompanied the Applicants' preliminary amendment dated August 12, 1996. Copies of the amendment and declaration are attached hereto as Exhibits 1 and 2.

**III. Proposed drawing correction.**

In paragraphs 3-4 and 6 of the Office action, the Patent Office requested the submission of a proposed drawing correction and the amendment of the brief description of the drawing to identify the two pages of Figure 10 as "10A and "10B". Attached hereto as Exhibit 3 is a proposed (informal) drawing correction. The Brief description of the drawing has been appropriately amended as well, at page 8, line 32. Accordingly, these objections may now be withdrawn. The Applicants wish to defer formal correction of the drawings and submission of a petition for photograph drawings until the application is allowed.

**IV. The Application is in compliance with the Sequence Rules.**

In paragraph 5 of the Office action and in a Notice to Comply, the Patent Office requested that the Application be amended to include the Figure 10 sequences therein, and to include cross-references to the Sequence Listing in the brief descriptions of Figs. 9B and 10. The Application has been so amended. The sequence listing amendment is accompanied by an appropriate

statement confirming that no new matter has been introduced into the application. Accordingly, this objection may properly be withdrawn.

**V. The Applicants request deferral of the requirement for a Budapest Treaty declaration.**

In paragraph 7 of the Office action, the Patent Office rejected claims 6 and 7 under 35 U.S.C. § 112, first paragraph, alleging that access to biological deposit material was required to use the claimed invention. As indicated in the specification at p. 39, the biological deposit was made pursuant to the provisions of the Budapest Treaty. A statement confirming the availability of the deposit is filed herewith, rendering this rejection moot.

The Budapest Treaty declaration filed herewith is intended solely to expedite prosecution, and is not intended as an admission that the deposited plasmid is required to satisfy § 112, first paragraph.

**VI. The rejection of claim 1 under § 112, first paragraph, should be withdrawn.**

In paragraph 8 of the Office action, the Patent Office rejected claim 1, alleging that the full scope of the claim was not enabled by the specification:

Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for polynucleotides [sic: encoding?] all polypeptides that bind the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The scope of claim 1 encompasses polynucleotides from any source that encode polypeptides that bind specifically to the Flt4 receptor. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the encoded proteins

are recited in the instant claims. There is no guidance provided by the specification to select those encompassed polynucleotides that encode proteins that specifically bind the Flt4 receptor with the exception of those teachings which support the subject matter indicated as enabled. There is no guidance to predict *a priori* whether any protein would bind the receptor *without some information on the structure of the protein*, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention.

(Office action at p. 5.)

The Applicants traverse-in-part and amend-in-part.

- A. The amendments to claim 1 overcome the factual bases for the Patent Office's rejection.

Claim 1 has been amended such that it no longer encompasses every polynucleotide that encodes all polypeptides that bind the Flt4 receptor. The Applicants have adopted the Examiner's suggestion to include additional limitations relating to the structure of the encoded protein.

In particular, amended claim 1 is directed only to polynucleotides that encode polypeptides that: (1) have a VEGF-homologous domain defined by eight conserved cysteines that are common to the PIGF/PDGF/VEGF family of polypeptides (see Fig. 10, positions 103, 130, 136, 139, 140, 147, 184, and 186); and (2) are capable of binding with *high affinity* to the *extracellular domain of human Flt4*. Thus, claim 1 has been further limited with respect to the source (human) and the domain (extracellular) of the binding partner; the nature (high affinity) of the binding reaction; and the type of polypeptide encoded (polypeptides which possess homology to a core portion of VEGF that is definable by eight conserved cysteines).

In addition, claim 1 now contains a significant structural limitation relating to the sequence of the claimed polynucleotide, namely, that the polynucleotide is sufficiently similar to the exemplified SEQ ID NO: 32 such that it will hybridize to the non-coding strand complementary to SEQ ID NO: 32 under specified hybridization conditions. The specified hybridization conditions

are those that were successfully employed in Example 10 to screen a PC-3 cell cDNA library to clone VEGF-C cDNA. (See specification at pp. 26-27.)

The scope of amended claim 1 is commensurate with the teachings in the application. Because of the hybridizing limitation, claim 1 reads only on those polynucleotides that can be identified via a routine hybridization screening assay that has been taught and successfully performed in the present application. Moreover, there is guidance in the specification that Flt4 ligands of the invention contain homology to VEGF (see, e.g., specification at p. 11, lines 11-13, and Figs. 10A-10B), and claim 1 has been appropriately limited in this manner. The specification teaches Flt4 binding assays that are useful to determine whether an encoded polypeptide binds Flt4.

In fact, subsequent hybridization experiments, using the human VEGF-C cDNA as a probe, were successfully performed to isolate VEGF-C-encoding cDNAs of mouse and quail. (See Declaration Under 37 C.F.R. §1.132 of Dr. Kari Alitalo at ¶¶ 10-18.) The identity of the encoded proteins was confirmed by receptor binding and stimulation studies. (*Id.*) This evidence that the specification enables one to isolate non-human VEGF-C-encoding cDNAs and confirm their identity in the receptor binding studies refutes the basis for the Patent Office's rejection.

For all of these reasons, claim 1 as amended is commensurate in scope with the teachings in the application, and the rejection under §112, first paragraph, should be withdrawn.

**B. The Borg reference cited by the Patent Office does not support the rejection.**

The Patent Office also cited a Borg publication in support of its rejection:

There is no guidance provided by the state of the art to select ligands to make the invention; despite intense research in this area, Borg et al. (reference C7) teach that no known ligands for the Flt4 receptor were known at the time of the invention.

(Office action at pp. 5-6.)

Reliance upon Borg is misplaced, because the Applicants do not rely upon Borg to provide an enabling disclosure. The Applicants specification provides the enabling disclosure. As explained above, amended claim 1 is commensurate in scope with guidance provided in the specification.

- C. The legal authorities relied upon by the Patent Office do not support a rejection of claim 1.

The Patent Office cites several cases in support of its rejection of claim 1:

The amount of guidance required varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). See also *Genentech, Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

(Office action at p. 6.)

The *Soll* opinion relied upon by the Patent Office is distinguishable because the patent applicant in that case was attempting to claim more broadly than the original disclosure of the patent application, which disclosure gave no indication that the applicant regarded his invention as a generic one. See *In re Soll*, 38 U.S.P.Q. at 190. In the present case, claim 1 has been amended herein to claim more narrowly than the generic invention originally contemplated and claimed by the inventors.

The *Fisher* opinion relied upon by the Patent Office relates to patent applications filed in the 1949-1960 period, well in advance of the genetic engineering techniques that were available and known in the art at the time the present application was filed. See *In re Fisher*, 166 U.S.P.Q. at 19-20. Because the genetic engineering techniques known to those skilled in the art at the time of the present application drastically reduce experimentation relative to

the traditional techniques that were known at the time of *Fisher*, the *Fisher* opinion cannot properly be applied against the present case.

The Patent Office's reliance upon the *Genentech* decision also is misplaced, because the facts of that case are wholly dissimilar from the facts of the present case.

Initially, it should be observed that *Genentech* is a case wherein the Court analyzed whether a 1979 patent application satisfied the enabling disclosure requirement. See *Genentech Inc. v. Novo Nordisk A/S*, 42 U.S.P.Q.2d 1001, 1004 (Fed. Cir. 1997). The state of the art relating to recombinant DNA and proteins was in a state of relative infancy in 1979, as compared to the state of the art in the 1994-1996 time period during which the present series of applications were filed. Since the enabling disclosure requirement involves an analysis of whether an application enables one of ordinary skill in the art to make and use an invention, and since the skill in the art has advanced enormously since 1979, the *Genentech* opinion is of little relevance.

Moreover, *Genentech* involved a unique situation wherein a patentee re-filed a patent application with a wholly new claim, in an attempt to enjoin an alleged infringer. As characterized by the Federal Circuit, the unique factual circumstances were as follows: an unsolved problem in the art had been the difficulty of obtaining a human protein (hGH) from a precursor that contained added protein material; Genentech's specification taught a solution to the problem wherein hGH was expressed *without* the added material; yet Genentech was attempting, in its re-filed application, to "bootstrap" by claiming a wholly different solution to the problem, namely, expression via synthesis and processing of a cleavable fusion protein. *Genentech*, 42 U.S.P.Q.2d at 1005. Genentech's specification contained "no disclosure of any specific starting material or of any of the conditions under which [the claimed] process can be carried out." *Id.* In these circumstances, the Court found Genentech's patent invalid.

In contrast, the present application teaches the procedures necessary to identify polynucleotides according to claim 1. For example, the present application teaches hybridization assays to identify candidate polynucleotides from other cDNA libraries; expression techniques for expressing polypeptides; and screening techniques to identify those polypeptides that bind to the extracellular domain of human Flt4.

The final case relied upon by the Patent Office, *In re Wands*, actually *supports*, rather than negates, a conclusion of enablement. The *Wands* opinion stands for the proposition that "Enablement is not precluded by the necessity for some experimentation such as routine screening. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). In *Wands*, the Federal Circuit reversed as improper a rejection under §112, first paragraph. The Court recognized that practitioners in the pertinent molecular biological art were prepared to perform multiple screening experiments of "negatives" in order to identify one "positive." *Wands*, 8 U.S.P.Q.2d at 1406. Moreover, for the purposes of evaluating whether experimentation is "undue," the Federal Circuit recognized that "an experiment" was not simply defined by the screening of a single clone, but rather, by a larger process that can involve producing and screening several clones:

Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics.

*In re Wands*, 8 U.S.P.Q.2d at 1407.

As explained more fully in Section VII, below, the present specification enables the present claims, under the standards established in *Wands*.



**VII. The rejection of claims 18-25 under §112, first paragraph, should be withdrawn.**

In paragraph 9 of the Office action, the Patent Office rejected claims 18-25, alleging that the full scope of the claims was not enabled by the specification:

Claims 18-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for the scope of polynucleotides commensurate with the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at pp. 6-8.)

The Applicants traverse-in-part and amend-in-part.

A principal basis for the rejection relates to the alleged scope of encoded-polypeptides encompassed by the claims:

The breadth of claims 18-25 encompasses polynucleotides encoding all active fragments of the protein of SEQ ID NO:33, or those that are 23 kDa or 32 kDa in size. Claim 21 recites the limitation that the fragment comprises SEQ ID NO:13, but this claim encompasses polypeptides in which SEQ ID NO:13 is the only sequence derived from the protein of SEQ ID NO:33. The only guidance offered by the specification to those fragments that may make the invention is provided at page 11 where at least residues 1-120 of SEQ ID NO:33 are taught to be required for activity by comparison to PDGF (*infra*).

(Office action at pp. 6-7.)

The Applicants respectfully submit that the breadth of the claims are commensurate in scope with the guidance in the specification.

- A. There is no undue experimentation involved in synthesizing the polynucleotides (or the encoded polypeptides) within the scope of the claims.**

By providing the amino acid sequence set forth in SEQ ID NO: 33, the specification enables one skilled in the art to make essentially any polypeptide comprising a portion of SEQ ID NO: 33, and enables one to make essentially any polynucleotide coding sequence for such polypeptide. For example, such polynucleotides may be synthesized using automated synthesizers or using recombinant techniques (e.g., using polynucleotides of the invention and/or variants thereof obtained by site-directed mutagenesis).

- B. There is no undue experimentation involved in screening polypeptides for the abilities to bind Flt4 or stimulate Flt4 phosphorylation.**

Claim 18 is limited to nucleic acids that comprise a sequence encoding a polypeptide comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4. This limitation is commensurate in scope with the teachings in the application, because the specification teaches that encoded polypeptides are Flt4 ligands, and teaches Flt4 binding assays (and phosphorylation assays) to determine whether a polypeptide is capable of binding to Flt4 receptor tyrosine kinase (and whether the peptide is capable of stimulating Flt4 autophosphorylation). (See, e.g., Examples 4-5.) Such assays are the "routine screening" type of assay contemplated by the Federal Circuit in the *Wands* opinion.

- C. The specification provides guidance for identifying portions of SEQ ID NO: 33 effective to permit Flt4 binding.**

The specification provides significant guidance for determining portions of SEQ ID NO: 33 that are effective to permit Flt4 binding. For example, although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the first 33 amino acids are not critical for Flt4 binding. (See, e.g., p. 23, lines 5-10, and p. 25, line 27, to p. 26, line 6, which teach that a mature form of VEGF-C lacks the first 33 residues of SEQ ID NO: 33.)

The specification further teaches that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage that produces a mature, naturally occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. (Specification at p. 5, lines 27-31.) There is guidance that the observed ~23 kD polypeptide exemplified in the application is likely to represent the VEGF-homologous domain of VEGF-C, and that the carboxy-terminal sequences that contain cysteine motifs reminiscent of a Balbiani ring 3 protein are cleaved off. (Specification at p. 11, lines 4-23.) At page 11, lines 33-35, attention is drawn to the probable importance of eight conserved cysteine residues of VEGF-C, which correspond to residues 29, 54, 60, 63-64, 71, 107, and 109 of SEQ ID NO: 33. (See Figure 10 and the amended Sequence Listing filed herewith.)

Additionally, the specification outlines a protocol for defining that portion of SEQ ID NO: 33 which corresponds with the naturally-occurring Flt4 ligand. (See pp. 29-30.) Furthermore, the specification provides guidance to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. (Specification at p. 30, lines 6-17.) The Declaration Under 37 C.F.R. §1.132 of Dr. Kari Alitalo filed herewith provides evidence that such procedures were successful in further characterizing the natural processing of VEGF-C and in identifying VEGF-C fragments that are capable of binding Flt4. (See ¶¶ 6-9.)

Collectively, these teachings serve to both provide guidance for predicting the portions of SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.

- D. The Patent Office's reliance upon Heldin to support its rejection is improper.

The Patent Office cited a Heldin publication in support of its rejection:

Heldin et al. (reference C40), however, teach the criticality of structures throughout the corresponding region of PDGF, such as extended loop structures (Figure 2), disulfide bonds involving residue 1 (page 249, column 1), and specific residues including those up to residue 154 (*loc. cit.*). Moreover, comparison with PDGF is of limited predictive value, because the stereo-specific interaction required between VEGF-C and its receptor are different than those between PDGF and its receptor as evidenced by the fact that the two ligands do not bind the same receptors. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Recitation that the encoded proteins must be 23 or 32 kDa in size does not provide significant additional guidance or limitation to the scope of the claims, because this limitation does not exclude the presence of sequences unrelated to VEGF-C within the polypeptide, nor does it exclude various post-translational modifications known to profoundly influence the apparent molecular weight without affecting the primary sequence of the polypeptide. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. In re Fisher, 166 USPQ 18 (CCPA 1970).

(Office action at p. 7.)

The Applicants agree that comparison with PDGF, *by itself*, would be of limited predictive value. However, as outlined above in Section C, comparison with PDGF is merely one of many factors taught in the specification for predicting which fragments bind the receptor; and the application teaches routine screening to determine which fragments *actually* bind the receptor.

- E. The Patent Office's suggestion that one skilled in the art must test every fragment of SEQ ID NO: 33 is incorrect.

Underlying the Patent Office's rejection is the implicit assumption that the experimentation involved to practice the invention would require the screening of every possible fragment of SEQ ID NO: 33:

The vast amount of experimentation required to test all the encompassed fragments is an additional factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

(Office action at pp. 7-8.)

As an initial matter, the Applicants wish to clarify that the claims as written are directed to polynucleotides comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to Flt4. The "testing" of polypeptide fragments for Flt4 binding determines whether polynucleotides encoding the fragments fall within the claims. Therefore, to the extent the claims have been interpreted as "encompassing" both binding and non-binding fragments, the Patent Office has improperly ignored a limitation of the claims.

Second, the Patent Office's assumption that one must test "all" fragments improperly ignores the significant guidance in the specification with respect to those portions of SEQ ID NO: 33 that are effective to permit binding to Flt4. This guidance drastically reduces the number of fragments that one would select for screening.

Moreover, the Patent Office's suggestion that it is necessary to test all fragments of SEQ ID NO: 33 ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that portion of SEQ ID NO: 33 that is effective for binding, and

that portion which is not.<sup>2</sup> An artisan of ordinary skill also understands techniques for accelerating a screening process,<sup>3</sup> and techniques for screening multiple polypeptides *simultaneously*. Thus, the examiner's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

- F. The basis for rejection is moot with respect to the Applicants new claims 30-36.

New claims 30-36 contain additional limitations (relative to rejected claims 18-25) that characterize portions of SEQ ID NO: 33 which are effective to permit Flt4 binding, and which are encoded by the claimed polynucleotide. These additional limitations render inapplicable the bases for rejection that the Patent Office alleged against claims 18-25. Evidence in support of the patentability of these claims is provided in paragraphs 6-9 of the Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo filed herewith.

- VIII. The rejection of claims 3-5, 11, 13, and 17-25 under §112, second paragraph, should be withdrawn.

In paragraph 10 of the Office action, the Patent Office rejected claims 3-5, 11, 13, and 17-25 under 35 U.S.C. §112, second paragraph, alleging that these claims were indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention.

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<sup>2</sup> For example, a determination that a polypeptide comprising residues 1-120 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 121-317 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

<sup>3</sup> For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 1-100, 1-110, 1-120, 1-130, 10-120, 20-120, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (1-130, 1-129, 1-128, 1-127, 1-126 . . .), to more rapidly identify effective portions for binding Flt4.

The Patent Office relied upon a Human Genome Sciences publication in support of its rejection:

SEQ ID NO:32 encodes a protein whose amino terminus is indicated as residue 1, and the specification teaches that the amino terminal of a 23 kDa protein expressed from the polynucleotide has the amino terminus shown in SEQ ID NO:13; however, Human Genome Sciences, Inc. disclose DNA encoding a similar sequence (99% global identity using the Smith-Waterman algorithm with 100% identity to the instantly claimed protein in the amino terminus through the instant residue 8) whose amino terminus is indicated as residue -8 of the instant protein. It would have been understood in the art that a disclosure of a particular residue as "residue 1" would have meant that this residue was the amino terminus of the mature polypeptide; however, it was unclear which residue corresponds to the amino terminus of the encoded polypeptide, making the designation of a particular amino acid residue as a "residue 1" indefinite. Although Human Genome Sciences was published after the effective filing date, the publication is used to show that the instant claims were indefinite at the time of filing. MPEP 2124 citing *In re Glass*, 492 F.2d 1228, 1232 n. 6., 181 USPQ 31, 34 n.6 (CCPA 1974).

(Office action at p. 8.)

Clarification is in order.

- A. The present application teaches that amino acid 1 is the threonine that is the 34th residue of SEQ ID NO: 33.

The Patent Office is correct that the specification teaches that the amino terminal amino acid of a 23 kDa protein expressed from a polynucleotide comprising SEQ ID NO: 32 has the amino terminus shown in SEQ ID NO: 13. This amino terminus corresponds with the 34th residue in SEQ ID NO: 33. As explained in the Statement Pursuant to 37 C.F.R. §1.825 filed herewith, the Sequence Listing has been amended herein to reflect the fact that this threonine residue represents the amino terminus of a mature VEGF-C protein.

The amino terminus taught in the present application reflects the results of amino acid sequencing of a purified VEGF-C protein secreted from a human cell line. (See Specification at pp. 21-23 (Example 5).) Thus, the

Applicants' asserted amino terminus is based upon the scientific characterization of a secreted human protein.

- B. Human Genome Sciences did not establish the amino terminus of a mature VEGF-C protein, and the cited publication is a mere guess that Human Genome Sciences later withdrew.

Apparently, the Patent Office relies upon Human Genome Science's International Patent Publication WO 95/24473 (hereinafter "HGS1") in support of its rejection. As set forth below, the HGS1 publication contains no sound scientific data to render indefinite the present claims or to call into question the Applicants' determination of the correct amino terminus of a mature VEGF-C protein.

The HGS1 publication teaches a "VEGF2" polypeptide "comprising 350 amino acids residues of which *approximately* the first 24 amino acids represent the leader sequence." (HGS1 at p. 4 (emphasis added).) The HGS1 publication does not base its determination of a "mature" 326 amino acid polypeptide on any scientific data. In fact, the only purported expression studies in the HGS1 publication were *in vitro* expression of PCR-amplified portions of cDNAs. (HGS1 at pp. 28-29.) The *in vitro* expression machinery employed would not necessarily process the expressed protein, and the HGS authors do not even report any analysis of the amino terminus of this *in vitro* protein. Because the splice site in the HGS1 publication is pure speculation, the authors assert only that "the first 24 amino acids residues *are likely to be leader sequence* . . . ." (HGS1 at p. 5 (emphasis added).)<sup>4</sup>

In fact, International Patent Publication No. WO 96/39515 (hereinafter "HGS2"), a later publication by a different Human Genome Sciences authorship entity, seems to *refute* the definition of a "mature" amino terminus that is proffered in HGS1. More particularly, HGS2 alleges that "VEGF2 contains an open reading frame encoding a protein of 419 amino acid residues

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<sup>4</sup> Moreover, HGS1 fails to identify a VEGF2 binding partner and fails to demonstrate a VEGF2 biological activity.



of which approximately the first 23 amino acid residues are the putative leader sequence such that the mature protein comprises 396 amino acids...." (HGS2 at p. 7.) Thus, more than one year after HGS1, the Human Genome Sciences scientists have apparently retracted or contradicted the teachings in HGS1 relating to a proper definition of "mature" VEGF2. HSG2, like HGS1, contains no scientific study to define a true "mature" VEGF2.

Thus, a careful scientific analysis reveals that the present application properly defines an amino terminus for a mature VEGF-C protein, based on sequencing studies of a VEGF-C protein that is actually expressed in a human cell line. The multiple, different amino termini alleged by Human Genome Sciences in its publications are mere speculation, unsupported by scientific evidence. Because the Applicants' asserted amino terminus is scientifically supported and the Human Genome Sciences purported amino termini are mere speculation, the Patent Office's indefiniteness rejection of claims 3-5, 11, 13, and 17-25 should be withdrawn.

**IX. The rejection of claims 3-5, 11, 13, and 17-25 under §112, second paragraph, should be withdrawn.**

In paragraph 11 of the Office action, the Patent Office rejected claims 11 and 13, under 35 U.S.C. §112, second paragraph, alleging that these claims were indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention:

Specifically, the term "approximately" in claims 11 and 13 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

(Office action at p. 9.)

Solely for the purpose of expediting allowance, the Applicants have amended claim 11 to remove the allegedly indefinite term. Claim 13 has been canceled

herein. These amendments render this rejection moot. The rejection should therefore be withdrawn.

**X. Comments concerning the Examiner's statement of reasons for the indication of allowable subject matter.**

In paragraph 13 of the Office action, the Examiner commented as follows about the state of the art:

The following is a statement of reasons for the indication of allowable subject matter: polynucleotides encoding the instantly claimed ligand appear to be novel over the prior art of record. Borg et al. (reference C7), for example, disclose that the ligand for Flt4 was not known in the art around the time of invention. Closest prior art is a DNA with about 99% identity to the claimed polynucleotide (Human Genome Sciences, Inc. reference B1), but the publication date antedates the effective filing date of the instant application. Other relevant prior art made of record below discloses a series of expressed sequence tags (ESTs) with high identity to large regions of the Flt4 ligand cDNA. The probable identity of these ESTs was not disclosed, and without the benefit of hindsight, the artisan at the time of invention would not have been motivated to use these ESTs to make the claimed invention. It was not known that these ESTs encoded a receptor ligand, nor was it known that these ESTs were nearly identical to the Flt4 ligand cDNA at the time of invention. In fact, the only EST posited to encode a particular protein was taught to encode a Balbiani ring protein (Hillier et al., EST-STS Accession No. T81690), hardly giving motivation to use the EST to find the claimed invention.

(Office action at pp. 9-10.)

The Applicants first wish to clarify that the publication date of reference B1 does *not* antedate the effective filing date of the instant application. It is apparent from the context of the Office action that this is what the Examiner intended.

Moreover, the Applicants respectfully submit that all of the claims in the present application would be patentable over reference B1, even if that reference (or a counterpart U.S. patent) constituted statutory prior art.

For example, claim 1 is directed to a host cell transformed or transfected with a polynucleotide of the invention, wherein the host cell expresses a polypeptide encoded by said polynucleotide, *said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)*. Reference B1 does not even suggest to try to recombinantly express a polypeptide lacking domains having BR3P cysteine motifs. Even if such a suggestion existed, there would be no reasonable expectation from reference B1 that such a polypeptide would bind Flt4. In fact, there is no recognition in reference B1 that any polypeptide binds Flt4. Thus, the subject matter of claim 1 is novel and unobvious over reference B1. It will be apparent that similar reasoning supports the novelty and nonobviousness of host cell claims 3, 4, 5, 7, 25-29, and 35, and of method claims 35-38.

Claim 18 is directed to a nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to Flt4, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, *said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein*. It is totally unexpected from reference B1 that a nucleic acid that encodes a portion of SEQ ID NO: 33 that lacks the recited BR3P encoding sequences still encodes a polypeptide that is capable of binding Flt4. These unexpected properties support the unobviousness of claim 18 and claims that depend therefrom. Similar considerations support the unobviousness of claims 33-35 over reference B1.

Thus, all of the pending claims would remain patentable over reference B1, even if this reference were statutory prior art.

XI. Prosecution has been suspended in a related application.

Pursuant to 37 C.F.R. §1.56, the Applicants wish to apprise the Examiner that prosecution has been suspended in a parent application (U.S.S.N. 08/510,133) "because a reference relevant to the examination . . . may soon become available." As set forth in Section X, above, if the reference is a U.S. patent counterpart to reference B1, the reference should not prevent allowance of the present application. All of the pending claims are directed to subject matter that is patentably distinct from anything disclosed or suggested in reference B1.

CONCLUSION

For the foregoing reasons, the applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification, and allowance of claims 1, 3-5, 7, 11, and 18-38.

Respectfully submitted,

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## **APPENDIX OF CLAIMS**

1. (Twice amended) A host cell transformed or transfected with a polynucleotide encoding a polypeptide that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase, wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

### **2. [CANCELED]**

3. (Twice amended) A host cell transformed or transfected with a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

4. (Amended) A host cell according to claim 3 wherein said nucleic acid comprises the sequence shown in SEQ ID NO: 32.

5. (Twice amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising a nucleic acid that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

### **6. [CANCELED]**

7. (Amended) A host cell comprising plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses a polypeptide encoded by said plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

8. [CANCELED]

9. [CANCELED]

10. [CANCELED]

11. (Twice amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.

12. [CANCELED]

13. [CANCELED]

14. [CANCELED]

15. [CANCELED]

16. [CANCELED]

17. [CANCELED]

18. (Amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

19. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase.

20. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

21. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.

26. A host cell according to claim 1 that expresses a naturally occurring VEGF-C protein encoded by said polynucleotide.

27. A host cell according to claim 1 that expresses a human VEGF-C protein encoded by said polynucleotide.

28. A host cell according to claim 27, wherein said host cell expresses said polynucleotide and produces a mature human VEGF-C protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to a nucleotide sequence that encodes said polypeptide.

30. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes a VEGF-homologous portion of SEQ ID NO: 33 and excludes the portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide consisting of a continuous portion of the sequence shown in SEQ

ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

37. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell.



SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari  
Joukov, Vladimir
- (ii) TITLE OF INVENTION: RECEPTOR LIGAND
- (iii) NUMBER OF SEQUENCES: 45
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(C) CITY: Chicago  
(D) STATE: Illinois  
(E) COUNTRY: United States of America  
(F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: 08/585,895  
(B) FILING DATE: 12-JAN-1996  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 08/510,133  
(B) FILING DATE: 01-AUG-1995
- (viii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 08/340,011  
(B) FILING DATE: 14-NOV-1994
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Gass, David A.  
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(C) REFERENCE/DOCKET NUMBER: 28967/33072
- (ix) TELECOMMUNICATION INFORMATION:  
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(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCTCTCGCT GTCTGTGCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCGC CGCTGTGCCT GCGACTGTGG CTCGCGCTGG 60  
GACTCCTGGA 70

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCGCGCGGTC ATCC 24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCC CATGACCCCA AC 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCGATGG ATCCTACTCG AAGCCGCTTT CTT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTTAGGTGA CACTATA 17

C3  
C4

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT

34

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Met Thr Pro Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp  
1 5 10 15  
Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg  
20 25 30  
His Arg Gln Glu Ser Gly Phe Arg  
35 40

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 60 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

C GCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs

C3  
cont.

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGCGCTGTG ATGTGCACCA

20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala His Tyr Asn Thr Glu Ile  
1 5 10 15  
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CNATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

C3  
cont.

Glu Glu Thr Ile Lys  
1 5

- (2) INFORMATION FOR SEQ ID NO:16:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 18 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATTYTCNGT

18

- (2) INFORMATION FOR SEQ ID NO:17:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 5 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Glu Ile Leu Lys  
1 5

- C3  
ent.*
- (2) INFORMATION FOR SEQ ID NO:18:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 22 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTGCTGTGCA GCACACTACA AC

22

- (2) INFORMATION FOR SEQ ID NO:19:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 19 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCNGTGTGTGT AGTGTGCTG

19

- (2) INFORMATION FOR SEQ ID NO:20:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 7 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGTAGTGT GCTGAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn  
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCATATAGG GAGACCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

C3  
cont.

(A) LENGTH: 219 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACATATAGG GAGACCCAAG CTGGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT 60  
GTGGTGGAAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG 120  
TGTCAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG 180  
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC 219

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC 18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATTT AGGTGACAC 19

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTCGCT GCAGC 25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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ent

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTCGA

20

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1140 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 37..1086

(ix) FEATURE:

- (A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 136..1086

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAACTC ATG ACT GTA CTC TAC CCA  
 Met Thr Val Leu Tyr Pro  
 -33 -30

54

GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA  
 Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln  
 -25 -20 -15

102

CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA  
 His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Thr Ile  
 -10 -5 1 5

150

C3  
 int.



AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT	198
Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp	
10	
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT	246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp	
25	
30	
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA	294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro	
40	
45	
50	
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG	342
Cys Val Ser Val Tyr Arg Cys Gly Cys Cys Asn Ser Glu Gly Leu	
55	
60	
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA	390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu	
70	
75	
80	
85	
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT	438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe	
90	
95	
100	
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA	486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg	
105	
110	
115	
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG	534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln	
120	
125	
130	
TGT CAG GCA GCG AAC AAG ACC TGC TGC ACC AAT TAC ATG TGG AAT AAT	582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn	
135	
140	
145	
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT	630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp	
150	
155	
160	
165	
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC	678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn	
170	
175	
180	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT	726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu	
185	
190	
195	
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC	774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys	
200	
205	
210	
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCA AAC GAG TGT GGG GCC AAC	822
Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn	
215	
220	
225	
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC	870
Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys	
230	
235	
240	
245	
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA	918
Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr	
250	
255	
260	
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA	966
Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln	
265	
270	
275	

C3  
unf.

ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT	1014
Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys	
280 285 290	
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA	1062
Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser	
295 300 305	
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT	1116
Tyr Trp Lys Arg Pro Gln Met Ser	
310 315	
TTTCTATTAT GGAAAACTGT GTTG	1140

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 350 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu  
 -33 -30 -25 -20  
 Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser  
 -15 -10 -5  
 Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu  
 1 5 10 15  
 Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro  
 20 25 30  
 Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn  
 35 40 45  
 Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys  
 50 55 60  
 Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu  
 65 70 75  
 Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys  
 80 85 90 95  
 Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser  
 100 105 110  
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu  
 115 120 125  
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr  
 130 135 140  
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp  
 145 150 155  
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His  
 160 165 170 175  
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys  
 180 185 190

C3  
cont.

-50-

Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu  
195 200 205  
Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro  
210 215 220  
Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys  
225 230 235  
Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys  
240 245 250 255  
Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly  
260 265 270  
Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr  
275 280 285  
Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val  
290 295 300  
Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser  
305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CG3  
cont.  
TGAGTGTATTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCAACCCACATCT

22

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 196 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Arg Thr Trp Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala  
1 5 10 15  
His Ala Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Leu Ile Glu Arg  
20 25 30  
Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu  
35 40 45  
Glu Ile Asp Ser Val Gly Ala Glu Asp Ala Leu Glu Thr Ser Leu Arg  
50 55 60  
Ala His Gly Ser His Ala Ile Asn His Val Pro Glu Lys Arg Pro Val  
65 70 75 80  
Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Ile Pro Ala Val Cys  
85 90 95  
Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro  
100 105 110  
Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg  
115 120 125  
Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg  
130 135 140  
Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys  
145 150 155 160  
Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu  
165 170 175  
Cys Ala Cys Ala Thr Ser Asn Leu Asn Pro Asp His Arg Glu Glu Glu  
180 185 190  
Thr Asp Val Arg  
195

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 241 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg  
1 5 10 15  
Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met  
20 25 30  
Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu  
35 40 45  
His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met  
50 55 60

Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg  
65 70 75 80  
Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu  
85 90 95  
Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp  
100 105 110  
Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln  
115 120 125  
Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr  
130 135 140  
Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg  
145 150 155 160  
Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu  
165 170 175  
Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser  
180 185 190  
Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val  
195 200 205  
Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg  
210 215 220  
Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly  
225 230 235 240  
Ala

C3  
cont.

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 149 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly  
1 5 10 15  
Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly  
20 25 30  
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly  
35 40 45  
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu  
50 55 60  
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu  
65 70 75 80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro  
85 90 95  
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly  
100 105 110  
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys  
115 120 125  
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp  
130 135 140  
Ala Val Pro Arg Arg  
145

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 170 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly  
1 5 10 15  
Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly  
20 25 30  
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly  
35 40 45  
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu  
50 55 60  
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu  
65 70 75 80  
Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro  
85 90 95  
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly  
100 105 110  
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys  
115 120 125  
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro  
130 135 140  
Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys  
145 150 155 160  
His Leu Cys Gly Asp Ala Val Pro Arg Arg  
165 170

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 147 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu  
1 5 10 15  
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly  
20 25 30  
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln  
35 40 45  
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu  
50 55 60  
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu  
65 70 75 80  
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Leu Glu Cys Val Pro  
85 90 95  
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His  
100 105 110  
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys  
115 120 125  
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Cys Asp Lys  
130 135 140  
Pro Arg Arg  
145

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 191 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: not relevant
  - (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu  
1 5 10 15  
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly  
20 25 30  
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln  
35 40 45  
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu  
50 55 60

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Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65      70      75      80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85      90      95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100     105     110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115     120     125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130     135     140
Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145     150     155     160
Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165     170     175
Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180     185     190

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(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 215 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

C3  
cont.

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Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1      5      10      15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20     25     30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35     40     45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50     55     60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65      70      75      80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85      90      95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100     105     110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115     120     125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130     135     140

```



Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr  
 145 150 155 160  
 Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His  
 165 170 175  
 Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr  
 180 185 190  
 Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys  
 195 200 205  
 Arg Cys Asp Lys Pro Arg Arg  
 210 215

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 232 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu  
 1 5 10 15  
 Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly  
 20 25 30  
 Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln  
 35 40 45  
 Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu  
 50 55 60  
 Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu  
 65 70 75 80  
 Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro  
 85 90 95  
 Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His  
 100 105 110  
 Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys  
 115 120 125  
 Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val  
 130 135 140  
 Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Lys Ser Arg Tyr  
 145 150 155 160  
 Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp  
 165 170 175  
 Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys  
 180 185 190

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn  
 195 200 205  
 Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr  
 210 215 220  
 Cys Arg Cys Asp Lys Pro Arg Arg  
 225 230

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1997 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCCGCCCCGC CTCCTCAAAA AGCTACACCG ACGCGGACCG CGGCGGGCTC CTCCTCGCCG 60  
 CTCGCTTCAC CTCGCGGGCT CGGAATGCGG GGAGCTCGGA TGTCCGGTTT CCTGTGAGGC 120  
 TTTTACCTGA CACCCGCCGC CTTTCCCGCG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG 180  
 GGAACGCGGA GCCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG 240  
 GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGG GCCCGCGCCC 300  
 CCACCCCTGC CCCCGCCAGC GGACCGGTCC CCCACCCCGG GTCCTTCAC C ATG CAC 357  
 Met His  
 1  
 TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG 405  
 Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu  
 5 10 15  
 CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC 453  
 Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser  
 20 25 30  
 GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT 501  
 Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala  
 35 40 45 50  
 TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA 549  
 Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val  
 55 60 65  
 GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG 597  
 Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys  
 70 75 80  
 TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC 645  
 Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn  
 85 90 95

C3  
 cont.

CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	693
Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala His Tyr	
100 105 110	
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	
115 120 125	
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	
135 140 145	
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	837
Ala Thr Asn Thr Phe Phe Lys Pro Cys Val Ser Val Tyr Arg Cys	
150 155 160	
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG	885
Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr	
165 170 175	
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA	933
Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln	
180 185 190	
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA	981
Gly Pro Lys Pro Val Thr Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg	
195 200 205 210	
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA	1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Cys Val His Ser Ile Ile Arg	
215 220 225	
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC	1077
Arg Ser Leu Thr Ala Thr Leu Pro Cys Gln Cys Gln Ala Ala Asn Lys Thr	
230 235 240	
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT	1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala	
245 250 255	
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT	1173
Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp	
260 265 270	
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC	1221
Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr	
275 280 285 290	
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC	1269
Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro	
295 300 305	
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA	1317
His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys	
310 315 320 325	
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA	1365
Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr	
325 330 335	
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT	1413
Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn	
340 345 350	
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG	1461
Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Pro Gln Lys Cys	
355 360 365 370	

C 3  
cont.



**PATENT**  
**28967/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s):	)	Title: RECEPTOR LIGAND
	)	
Alitalo et al.	)	
	)	
Serial No: 08/585,895	)	Group Art Unit: 1801
	)	
Filed: January 12, 1996	)	Examiner: Lathrop, B.
	)	

**AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME**

*Assistant Commissioner for Patents  
Washington, D.C. 20231*

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111 and 1.115, including:  
(A) new pages 40-60 comprising a paper copy of a substitute Sequence Listing;  
(B) Exhibits 1,2 and 3;
2. Computer-readable copy of substitute Sequence Listing;
3. Statement Pursuant to 37 C.F.R. 1.825(a) and 1.825(b);
4. Declaration under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
5. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
6. Check in the amount of \$475.00 in payment of fee for extension of time; and
7. Check in the amount of \$360 in payment of fee for extra claims.

**CERTIFICATE OF MAILING (37 CFR 1.8)**

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on November 26, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass

1. **Small Entity Status**

- ☒ Small entity status has been established and is still effective.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)	FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY	
One Month		\$110.00		\$55.00
Two Months		\$400.00		\$200.00
Three Months		\$950.00	X	\$475.00
Four Months		\$1,510.00		\$755.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$475.00

- ☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid therefor of \$\_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$475.00

3. Fee for Claims

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	33	MINUS	20	13	X11 =	\$143	X22 =	\$
INDEP.	5	MINUS	3	2	X41 =	\$82	X82 =	\$
First Presentation of Multiple Dependent Claim					+ 135 =	\$135	+270 =	\$
TOTAL ADDITIONAL FEE						\$360	OR	\$

4. Method of Payment of Fees

Attached are checks in the amount of \$475 and \$360.

- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

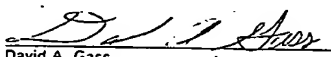
5. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By:   
David A. Gass  
Reg. No: 38,153

November 26, 1997



PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	)	Title: RECEPTOR LIGAND
Alitalo et al.	)	
Serial No: 08/585,895	)	Group Art Unit: 1801
Filed: January 12, 1996	)	Examiner: Lathrop, B.

AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111 and 1.115, including:  
(A) new pages 40-60 comprising a paper copy of a substitute Sequence Listing;  
(B) Exhibits 1,2 and 3;
2. Computer-readable copy of substitute Sequence Listing;
3. Statement Pursuant to 37 C.F.R. 1.825(a) and §1.825(b);
4. Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo;
5. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
6. Check in the amount of \$475.00 in payment of fee for extension of time; and
7. Check in the amount of \$360 in payment of fee for extra claims.

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David A. Gass

## DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed as Application Serial No. 08/583,895. I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

950674 Finland 13 February 1995  
(Application Serial Number) (Country) (Day/Month/Year Filed)

Priority Claimed  
☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number) (Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurs between the filing date of the prior application(s) and the national or PCT international filing date of this application:

05/140,011 14 November 1994 Pending  
(Application Serial Number) (Day/Month/Year Filed) (Status-Patented, Pending or Abandoned)  
05/510,133 01 August 1995 Pending  
(Application Serial Number) (Day/Month/Year Filed) (Status-Patented, Pending or Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)	Tyler B. Jacks (23,542)	Richard A. Scherer (30,990)	James J. Nagels (22,341)
Donald L. Bove (19,490)	Timothy J. Watson (26,348)	Anthony Nisano (30,920)	Richard M. La Berge (32,234)
Juan J. Murry (22,111)	Carl E. Moore, Jr. (28,487)	Christine A. Dufek (31,245)	Jeffrey W. Smith (32,455)
Allen H. Gorman (22,218)	Richard H. Anderson (28,326)	Karin D. Hegg (31,879)	Douglas C. Hochstetler (33,710)
Hans F. Souders (22,320)	Patrick D. Ernst (28,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Scheller (34,245)
Edward M. O'Toole (23,477)	James P. Zeller (28,491)	Donald J. Perchepin (32,167)	Robert M. Gorman (34,824)
Michael F. Bove (23,647)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	David A. Goss (38,133)

Send correspondence to: David A. Goss

FDN NAME PHONE NO STREET CITY & STATE ZIP CODE

Marshall, O'Toole, Gorman,  
Murry & Bove 312 474-6300 6700 East Tower  
Kari Alitalo 233 South Wacker Drive Chicago, Illinois 60608-6422

Full Name of Firm or Sole Inventor	Citizenship
Kari Alitalo	Finland
Residence Address - Street	Post Office Address - Street
Nyyrikintie 4A	Same
City (Zip)	City (Zip)
02100 Espoo	Same
Name or Country	Name or Country
FINLAND	Same
Date	Signature
June 6, 1996	[Signature]

See second page for additional inventor

See reverse for relevant rules & statutes

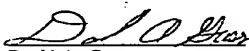




<b>Signed Asset Invoices, if any</b>	<b>Citizenship</b>
Vladimir Joutkov	Russia
<b>Residence Address - Street</b>	<b>Post Office Address - Street</b>
Topeliuksenkatu 32G8	Same
<b>City (Zip)</b>	<b>City (Zip)</b>
00290 Helsinki	Same
<b>State or Country</b>	<b>State or Country</b>
FINLAND	Same
<b>Date</b>	<b>Signature</b>
■ Aug. 6, 1996	■ V. Joutkov

PATENT  
28967/33072

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of:	)	I hereby certify that this paper is being
Alitalo et al.	)	deposited with the United States Postal
Serial No.: 08/585,895	)	Service as first class mail, postage
Filed: January 12, 1996	)	prepaid, in an envelope addressed to:
Title: RECEPTOR LIGAND	)	Assistant Commissioner for Patents
Art Unit: 1801	)	Washington, D.C. 20231, on this date:
Examiner: Lathrop, B.	)	Dated: <u>Nov. 26, 1997</u>
	)	
	)	David A. Gass
	)	Registration No. 38,153

DECLARATION OF BIOLOGICAL CULTURE DEPOSIT  
IN COMPLIANCE WITH BUDAPEST TREATY REQUIREMENTS

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, the undersigned, declare that:

1. I am an inventor of the subject matter of the above-identified patent application.
2. The plasmid designated FLT4-L, described in the specification of the above-identified application at pages 28-29 (and elsewhere), was deposited on 24 July 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, under the terms of the Budapest Treaty. This plasmid was assigned ATCC accession number 97231. A copy of the ATCC deposit receipt, confirming viability of the deposit, is attached hereto.

3. With respect to the permanence of the deposit, the ATCC is an official depository in accordance with the Budapest Treaty for the above-deposited material, and I affirm that, should the plasmid identified in paragraph 2 mutate, become non-viable, or be inadvertently destroyed, I will replace it for at least thirty (30) years from the date of the original deposit, or for at least five (5) years from the date of the most recent request for release of a sample, or for the enforceable life of any patent issued on the above-mentioned application, whichever period is longest.

4. With respect to availability of the plasmid identified in paragraph 2, I affirm that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if an enforceable patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent [MPEP §608.01 (p)], and (b) access to the deposit will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

5. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997  
Date

Kari Alitalo  
Kari Alitalo



## American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301) 331-5320 Telex: 890-055 ATCCNORTH • FAX: 301-770-2587

### BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

#### INTERNATIONAL FORM

#### RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

University of Helsinki  
Attention: Kari Alitalo  
Molecular/Cancer Biology Laboratory  
P.O. Box 21 (Haartmaninkatu 3)  
SF-00014, HELSINKI, FINLAND

Deposited on Behalf of: Kari Alitalo and Vladimir Joukov

Identification Reference by Depositor:

ATCC Designation

Plasmid, FLT4-L

97231

The deposit was accompanied by:    a scientific description    a proposed taxonomic description indicated above.

The deposit was received July 24, 1995 by this International Depository Authority and has been accepted.

#### AT YOUR REQUEST:

☒ We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested August 1, 1995. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

  
Annette L. Bade, Director, Patent Depository

Date: August 9, 1995

cc: Thomas C. Meyers



PATENT  
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	I hereby certify that this paper is
	)	being deposited with the United
Alitalo et al.	)	States Postal Service as first class
	)	mail, postage prepaid, in an
Serial No. 08/585,895	)	envelope addressed to: Assistant
	)	Commissioner for Patents,
Filed: January 12, 1996	)	Washington, D.C. 20231, on this
	)	date:
For: RECEPTOR LIGAND	)	
	)	Dated: <u>November 26, 1997</u>
Art Unit: 1801	)	
	)	
Examiner: Lathrop, B.	)	<u>David A. Gass</u>
	)	David A. Gass

STATEMENT PURSUANT TO 37 C.F.R. §1.825(a) and §1.825(b)

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I hereby state that the content of the paper and computer-readable forms of the substitute Sequence Listing submitted herewith, for entry as part of the above-identified application, are the same as each other and do not introduce new matter into the disclosure of the application. All of the amendments embodied in the substitute Sequence Listing filed herewith find support in the application as originally filed.

SEQ ID NOs: 1-31 and 34-35 of the original and substitute Sequence Listings are identical. Therefore, no new matter has been introduced in these sequences.

SEQ ID NOs: 36-43 have been added to the substitute Sequence Listing pursuant to instructions from the Patent Office to include sequences therein that are depicted in Figure 10 of the application. Because these eight sequences all find support in Figure 10 as originally filed, they do not introduce

new matter. Appropriate cross-references to SEQ ID NOs: 36-43 have been included in the brief description of the drawing.

SEQ ID NOs: 32-33 of the original and substitute Sequence Listings are identical. However, the amino acid numbering of these sequences has been amended in the substitute sequence listing by identifying the 34th residue in the substitute sequence listing as residue 1. (In the original sequence listing, the 33rd residue was identified as residue 1.) This amendment finds support throughout the specification as originally filed. For example, the description of the amino terminus of a mature form of VEGF-C is found in the specification at p. 23, lines 5-10, and is confirmed at page 25, line 27, to page 26, line 6 (from which it is apparent that the first 46 codons comprise 33 "signal sequence" residues and 13 amino acid residues of a secreted Flt4 ligand). From these excerpts of the specification that identify the amino terminus of a mature VEGF-C protein, it is clear that the residues of SEQ ID NO: 33 as originally filed were misnumbered by one residue. Because the error and its proper correction are apparent from the specification as originally filed, the corrections to SEQ ID NOs: 32-33 do not introduce new matter.

SEQ ID NOs: 44-45 of the substitute Sequence Listing depict a 1997 base pair nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the ATCC and cross-referenced at pp. 28-29 of the patent application as filed. These sequences are inherent properties of the deposited plasmid and thus find support in the deposited plasmid itself. See *Kennecott Corp. v. Kyocera International Inc.* 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date); *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985); see also Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo (filed herewith) at ¶¶ 2-5.


In accordance with 37 C.F.R. §1.68, I hereby declare that the foregoing statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

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233 South Wacker Drive  
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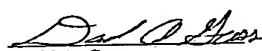
November 26, 1997

  
David A. Gass  
Registration No. 38,153



PATENT  
28967/33072

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of:	)	I hereby certify that this paper is being
	)	deposited with the United States Postal
Alitalo et al.	)	Service as first class mail, postage
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Serial No.: 08/585,895	)	Assistant Commissioner for Patents
	)	Washington, D.C. 20231, on this date:
Filed: January 12, 1996	)	
	)	Dated: <u>Nov 26, 1997</u>
Title: RECEPTOR LIGAND	)	
	)	
Art Unit: 1801	)	
	)	
Examiner: Lathrop, B.	)	David A. Gass
	)	Registration No. 38,153

**DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO**

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated May 28, 1997, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

**Isolation of VEGF-C protein and cDNA**

2. The present invention relates generally to a protein ligand for Flt4 receptor tyrosine kinase (VEGFR-3), which our research team has designated "VEGF-C." As taught in Example 14 of the patent application, VEGF-C also stimulates KDR/Flk-1 receptor tyrosine kinase (VEGFR-2). Our research team purified a VEGF-C protein that we discovered in conditioned media from a PC-3 prostatic adenocarcinoma cell line. We demonstrated that this protein bound to the extracellular domain of Flt4 and stimulated Flt4 phosphorylation. (See the patent application at Examples 4-5, for example.) Using SDS polyacrylamide gel electrophoresis, the VEGF-C protein was originally determined to have a molecular weight of about 23 kilodaltons. This measurement is in good



agreement with subsequent measurements of VEGF-C that we have recombinantly expressed in multiple cell lines, where we have determined the molecular weight to be about 21-23 kD.)

3. We sequenced the amino terminus of this purified VEGF-C protein as taught in the patent application in Example 5. (See especially p. 23.) I hereby reaffirm that our sequencing data from this protein is correctly reported in the patent application at p. 23 and in SEQ ID NO: 13.

4. As taught in Examples 6-10 of the patent application, we used the amino terminal amino acid sequence taught in the patent application to obtain a cDNA encoding VEGF-C. A plasmid containing the cDNA that is described in Example 11 of the patent application was deposited with the American Type Culture Collection and accorded ATCC accession number 97231.

5. The patent application describes a partial nucleotide sequence and a 350 amino acid open reading frame of the deposited VEGF-C cDNA. (See SEQ ID NOs: 32 and 33 of the patent application.) In the amendment filed herewith, these sequences have been amended such that the designation of residue "1" therein corresponds with the first residue of VEGF-C purified from PC-3 conditioned medium as described in the patent application. (See also paragraph 3, above.) Amended SEQ ID NOs: 32-33 are attached hereto as Exhibit A. Complete sequencing of the cDNA subsequently demonstrated that the translated open reading frame is actually 419 amino acids: it extends 69 codons upstream of what is reported in SEQ ID NO: 33. Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45. I shall use the term "prepro-VEGF-C" herein to refer to a polypeptide consisting of this 419 amino acid sequence.

6. As taught in the patent application (e.g., at p. 11), the carboxyl-terminal amino acid sequences encoded by the VEGF-C cDNA show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring 3 protein (BR3P) sequence that was

known in the art. (See Dignam and Case, *Gene*, 88:133-40 (1990); and Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990), both of record and cited in the patent application). The distinctive BR3P cysteine motifs (Cys-Xaa<sub>n</sub>-Cys-Xaa-Cys-Xaa-Cys, wherein Xaa is any residue and n is variable) occur at least four times in the carboxy-terminal portion of VEGF-C (see Cys residues in Exhibit B at positions 280, 291, 293, and 295; positions 304, 315, 317, and 319; positions 328, 339, 341, and 343; and positions 347, 358, 360, and 362).

**VEGF-C processing and determination of  
VEGF-C fragments that bind to Flt4.**

7. The Patent application teaches that the protein encoded by the VEGF-C gene is proteolytically processed, and teaches procedures to characterize this processing, such as analysis using antibodies and pulse-chase experiments. The application further teaches to screen truncated forms of VEGF-C (e.g., deletion fragments) to determine the portions of VEGF-C that are necessary to bind and stimulate Flt4. (See, e.g., pp. 29-30 of the patent application.) Using techniques such as those described at pp. 29-30 of the patent application and mutational analysis, our research team has extensively characterized the processing of human prepro-VEGF-C in mammalian cell lines.

A. Our results from pulse-chase experiments indicate that the apparent first proteolytic processing of human prepro-VEGF-C involves cleavage of a signal peptide of about 31 residues, leaving residues 32-419 (hereinafter "pro-VEGF-C"). Pro-VEGF-C has an apparent molecular weight of about 55-58 kD.

B. We next observed that pro-VEGF-C is cleaved, either intracellularly or at the cell surface, into polypeptides of about 29 kD and about 31-32 kD (when assessed by SDS-PAGE under reducing conditions). The ~32 kD polypeptide binds the extracellular domain of Flt4 receptor tyrosine kinase with high affinity. (See Example 13 of the patent application.) The ~32 kD polypeptide was purified with immunoaffinity chromatography using an anti-VEGF-C antibody. The amino-terminus of

this purified polypeptide was determined to correspond to position 32 of the sequence shown in Exhibit B. Thus, the ~32 kD polypeptide represents the amino-terminal product of this proteolytic cleavage. Sequencing of the ~29 kD polypeptide indicated that cleavage occurred after amino acid 227 of the 419 amino acid sequence depicted in Exhibit B. (Amino acid 227 corresponds to residue 125 of SEQ ID NO: 33 in the patent application (Exhibit A).) This carboxy-terminal fragment of about 29 kD presumably includes residues 228-419 of the sequence depicted in Exhibit B (residues 126-317 of SEQ ID NO: 33). Thus, the ~29 kD polypeptide includes all of the Balbiani ring 3 protein cysteine motifs of VEGF-C (see paragraph 6 above). These results indicate that polypeptide fragments of the sequences depicted in Exhibits A or B that lack any domain having cysteine motifs of a Balbiani ring 3 protein (e.g., that lack the ~29 kD carboxy-terminal fragment) remain capable of binding with the extracellular domain of Flt4.

C. We also have observed forms of VEGF-C that reflect further proteolytic processing at the amino terminus. For the purpose of this declaration, I shall collectively refer to forms of VEGF described below as "mature VEGF-C."

- i. As indicated in paragraph 3, above, VEGF-C isolated from conditioned medium of PC-3 cells has an amino terminus corresponding to amino acid 103 in Exhibit B (i.e., amino acid 1 of SEQ ID NO: 33 (Exhibit A)).
- ii. We have sequenced VEGF-C that was recombinantly expressed in 293-EBNA cells (as described in Example 11 of the patent application) and determined that the amino terminus of this form corresponds with position 112 of the sequence shown in Exhibit B (i.e., position 10 of SEQ ID NO: 33 (Exhibit A)).

8. Our research team modified the human VEGF-C cDNA to recombinantly produce a fragment consisting of amino acids 104-213 of the 419 amino acid polypeptide in yeast (i.e., residues 2-111 of SEQ ID NO: 33). This fragment was shown to bind Flt4 and stimulate phosphorylation of both Flt4 (VEGFR-3) and KDR (VEGFR-2). In another experiment, a fragment lacking residues 1-112 of the 419 amino acid polypeptide retained receptor binding activity.

9. Collectively, the experimental results described in the preceding paragraphs indicate that polypeptides lacking amino acids 1-112 and 214-419 of the 419 residue amino acid sequence shown in Exhibit B retain Flt4 binding and stimulating activities. Stated differently, we have experimental evidence to indicate that a polypeptide corresponding to positions 11-112 of SEQ ID NO: 33 will retain Flt4 binding and stimulating activities. Moreover, one skilled in the art understands from the patent application how to perform receptor binding and phosphorylation assays, to localize further the portion of SEQ ID NO: 33 that is required for activity.

**The application enables one to obtain  
VEGF-C-encoding cDNAs from non-human sources**

10. I infer from page 5 of the Office action that the Patent Office has rejected a claim of the application in part because of the lack of a claim limitation with respect to the source animal for VEGF-C. This section of the declaration provides evidence that the teachings in the patent application of a human VEGF-C cDNA, combined with the teachings that VEGF-C protein binds Flt4 (VEGFR-3) and VEGFR-2, enable one to obtain VEGF-C-encoding cDNAs from non-human sources.

11. To clone a murine VEGF-C cDNA, approximately  $1 \times 10^6$  bacteriophage lambda clones of a commercially-available 12 day mouse embryonal cDNA library (lambda EXlox library, Novagen, catalog number 69632-1) were screened with a radiolabeled fragment of human VEGF-C cDNA containing nucleotides 495 to 1661 of the nucleotide sequence shown in Exhibit B. One positive clone was isolated.

12. A 1323 bp *EcoRI/HindIII* fragment of the insert of the isolated mouse cDNA clone was subcloned into the corresponding sites of the pBluescript SK+ vector (Stratagene) and sequenced. The cDNA sequence of this clone was homologous to the human VEGF-C sequence reported herein, except that about 710 bp of 5'-end sequence present in the human clone was not present in the mouse clone.

13. For further screening of mouse cDNA libraries, a *HindIII-BstXI* (*HindIII* site is from the pBluescript SK+ polylinker) fragment of 881 bp from the coding region of the mouse cDNA clone was radiolabeled and used as a probe to screen two additional mouse cDNA libraries. Two additional cDNA clones from an adult mouse heart ZAP II cDNA library (Stratagene, catalog number 936306) were identified. Three additional clones also were isolated from a mouse heart 5'-stretch-plus cDNA library in  $\lambda$ gt11 (Clontech Laboratories, Inc., catalog number ML5002b). Of the latter three clones, one was found to contain an insert of about 1.9 kb. The insert of this cDNA clone was subcloned into *EcoRI* sites of pBluescript SK+ vector and both strands of this clone were completely sequenced, resulting in the nucleotide and deduced amino acid sequences shown in Exhibit C. It is expected that the mouse VEGF-C polypeptide depicted in Exhibit C is processed into a mature mouse VEGF-C protein, in a manner analogous to the processing of the human prepro-VEGF-C.

14. The foregoing results demonstrate the utility of human VEGF-C-encoding polynucleotides of the invention for identifying and isolating polynucleotides encoding other non-human mammalian VEGF-C proteins. Such identified and isolated polynucleotides, in turn, can be expressed (using procedures similar to those described in the patent application for human VEGF-C) to produce recombinant polypeptides corresponding to non-human mammalian forms of VEGF-C.

15. The identity of the mouse protein as VEGF-C was confirmed by recombinantly expressing the above-described mouse cDNA, and analyzing the expressed proteins.

A. The 1.8 kb mouse VEGF-C cDNA was cloned as an *EcoRI* fragment into the retroviral expression vector pBabe-puro containing the SV40 early promoter region [Morgenstern *et al.*, *Nucl. Acids Res.*, 18:3587-3595 (1990)], and transfected into the Bosc23 packaging cell line [Pearet *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 90:8392-8396 (1994)] by the calcium-phosphate precipitation method. For comparison, Bosc23 cells also were transfected with the previously-described human VEGF-C construct in the pREP7 expression vector. The expressed proteins were immunoprecipitated with polyclonal antibodies raised against mature human VEGF-C.

B. Immunoprecipitation of VEGF-C from media of transfected and metabolically-labelled cells revealed bands of approximately  $30\text{-}32 \times 10^3$  M<sub>r</sub> (a doublet) and  $22\text{-}23 \times 10^3$  M<sub>r</sub> in 12.5% SDS-PAGE. These bands were not detected in samples from nontransfected or mock-transfected cells. These results demonstrate that antibodies raised against human VEGF-C recognize the corresponding mouse protein.

C. For receptor binding experiments, 1 ml aliquots of media from metabolically-labelled Bosc23 cells were incubated with VEGFR-3 extracellular domain, covalently coupled to sepharose, for 4 hours at 4°C with gentle mixing. (See Examples 4 and 5 in the patent application.) The sepharose beads were washed four times with ice-cold phosphate buffered saline (PBS), and the samples were analyzed by gel electrophoresis as described in Joukov *et al.*, *EMBO J.*, 15:290-298 (1996).

D. Similar  $30\text{-}32 \times 10^3$  M<sub>r</sub> doublet and  $22\text{-}23 \times 10^3$  M<sub>r</sub> polypeptide bands were obtained in the receptor binding assay as compared to the immunoprecipitation assay. In additional experiments, mouse VEGF-C appeared to be a potent inducer of VEGFR-3 autophosphorylation, too. Thus, the putative mouse VEGF-C binds and stimulates human VEGFR-3, confirming its identity. The slightly faster mobility of the mouse VEGF-C polypeptides that was observed may be caused by the four amino acid

residue difference observed in sequence analysis (residues H88-E91).

Murine VEGF-C appeared to bind VEGFR-2 with lower affinity.

16. The human VEGF-C cDNA also was used to design probes for successfully isolating a quail VEGF-C cDNA from a quail cDNA library. A fragment of the human VEGF-C cDNA comprising nucleotides 495-1661 of Exhibit B was obtained by PCR amplification, cloned into the pCRII vector (Invitrogen) according to the manufacturer's instructions, and amplified. The insert was isolated by *Eco* RI digestion and preparative gel electrophoresis and then labelled using radioactive dCTP and random priming. A cDNA library made from quail embryos of stage E-4 in pcDNA-1 vector (Invitrogen) was then screened using this probe. About 200,000 colonies were plated and filter replicas were hybridized with the radioactive probe under reduced stringency conditions (washes at 42°C with a wash solution comprising 2x SSC/0.1% SDS). Nine positive clones were identified and secondarily plated. Two of the nine clones hybridized in secondary screening. The purified clones (clones 1 and 14) had approximately 2.7 kb *Eco* RI inserts. Both clones were amplified and then sequenced using the T7 and SP6 primers (annealing to the vector). In addition, an internal *Sph* I restriction endonuclease cleavage site was identified about 1.9 kb from the T7 primer side of the vector and used for subcloning 5'- and 3'- *Sph* I fragments, followed by sequencing from the *Sph* I end of the subclones. The sequences obtained were identical from both clones and showed a high degree of similarity to the human VEGF-C coding region. Subsequently, walking primers were made in both directions and double-stranded sequencing was completed for 1743 base pairs, including the full-length open reading frame.

17. The cDNA sequence obtained includes a long open reading frame and 5' untranslated region. The DNA and deduced amino acid sequences for the quail cDNA are set forth in Exhibit D. Studies performed with the putative quail VEGF-C cDNA have shown that its protein product is secreted from transfected cells and interacts with avian VEGFR-3 and VEGFR-2, further confirming the conclusion that the cDNA encodes a quail VEGF-C protein.

18. As shown in Exhibit E, the human, murine, and avian (quail) VEGF-C precursor amino acid sequences share a significant degree of conservation. This high degree of homology confirms the likelihood of success of attempts to isolate VEGF-C encoding sequences from other species, especially vertebrate species, and more particularly mammalian and avian species, using human VEGF-C-encoding polynucleotides taught in the patent application as probes and using standard molecular biological techniques. The identity of putative VEGF-C-encoding cDNAs is confirmed using receptor binding studies such as the studies described in the patent application.

Certification

19. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997  
Date

Jan Alitalo  
Kari Alitalo



# EXHIBIT A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAACTC	ATG ACT GTA CTC TAC CCA	54
	Met Thr Val Leu Tyr Pro	
	-33 -30	
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA		102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln		
	-25 -20 -15	
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA		150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile		
	-10 -5 1 5	
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT		198
Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp		
	10 15 20	
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT		246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp		
	25 30 35	
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA		294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro		
	40 45 50	
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG		342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu		
	55 60 65	
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA		390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu		
	70 75 80 85	
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT		438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe		
	90 95 100	
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA		486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg		
	105 110 115	
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG		534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln		
	120 125 130	
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT		582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn		
	135 140 145	
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT		630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp		
	150 155 160 165	
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC		678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn		
	170 175 180	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTG TGC AGA GCG GGG CTT		726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu		
	185 190 195	
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC		774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys		
	200 205 210	

200	205	210	
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn 215 220 225			822
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys 230 235 240 245			870
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr 250 255 260			918
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln 265 270 275			966
ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys 280 285 290			1014
CAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser 295 300 305			1062
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT Tyr Trp Lys Arg Pro Gln Met Ser 310 315			1116
TTTCTATTAT GGAAACTGT GTTG			1140

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu -33 -30 -25	
Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser -15 -10 -5	
Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu 1 5 10 15	
Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro 20 25 30	
Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn 35 40 45	
Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys 50 55 60	
Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu 65 70 75	
Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys 80 85 90 95	

Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser  
 100 105 110  
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu  
 115 120 125  
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr  
 130 135 140  
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp  
 145 150 155  
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His  
 160 165 170 175  
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys  
 180 185 190  
 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu  
 195 200 205  
 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro  
 210 215 220  
 Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys  
 225 230 235  
 Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys  
 240 245 250 255  
 Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly  
 260 265 270  
 Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr  
 275 280 285  
 Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val  
 290 295 300  
 Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser  
 305 310 315

EXHIBIT B

CCCGCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCTCGCC	60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCCGGTTT CCTGTGAGGC	120
TTTACCTGA CACCCGCGCG CTTTCCCGCG CACTGGCTGG GAGGGCGCCC TGCAAAAGTTG	180
GGAACGCGGA GCGCGGAGCC CGCTCCCGCC GCCTCCGGCT CGCCGAGGGG GGGTCGCGGG	240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC	300
CCACCCCTGC CCGCGCAGC GGACCGGTCC CCCACCCCGC GTCCTTCCAC C ATG CAC Met His 1	357
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu 5 10 15	405
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCG GCC TTC GAG TCC Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser 20 25 30	453
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GCG GAG GCC ACG GCT Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala 35 40 45 50	501
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val 55 60 65	549
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys 70 75 80	597
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn 85 90 95	645
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr 100 105 110	693
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln 115 120 125 130	741
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GCG AAG GAG TTT GGA GTC Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val 135 140 145	789
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys 150 155 160	837
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981

TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225	1029
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr 230 235 240	1077
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255	1125
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp 260 265 270	1173
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Gln Leu Asp Glu Glu Thr 275 280 285 290	1221
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro 295 300 305	1269
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320	1317
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415	1605
AGC TAAGATGTGA CTGTTTCCA GTTCATCGAT TTTCTATTAT GGAAACTGT Ser	1658
GTTCACACAG TAGAACTGTC TGTGAACAGA GAGACCCCTTG TGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCCTTCCTGA ACCATGTGGA TAACCTTACA GAAATGGACT GGAGCTCATC	1778
TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAACAGGCC AAGATTTTCC	1838
TCCTGTGATT TCCTTAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT	1898
GCATTCAITTT TTATAGCAAC AACRAATTGGT AAAACTCACT GTGATCAATA TTTTATATC	1958
ATGCCAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT	1997

# EXHIBIT C

## Mouse VEGF-C cDNA and deduced amino acid sequence

GCGGCGCGGT	CGACGCNAAA	GTTCGAGGCC	GCCGAGTCCC	GGGAGACGCT	CGCCAGGGG	60
GGTCCCGGG	AGGAAACCAC	GGGACAGGA	CCAGGAGAGG	ACCTCAGCCT	CACGCCCCAG	120
CTTGCGCCAG	CCAACGGACC	GGCTCCCTG	CTCCCGGTCC	ATCCACC	ATG CAC TTG Met His Leu 1	176
CTG TGC TTC TTG TCT CTG GCG TGT TCC CTG CTC GCC GCT GCG CTG ATC	Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala Leu Ile	5 10 15				224
CCC AGT CCG CGC GAG GCG CCC GCC ACC GTC GCC GCC TTC GAG TCG GGA	Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe Glu Ser Gly	20 25 30 35				272
CTG GGC TTC TCG GAA GCG GAG CCC GAC GGG GGC GAG GTC AAG GCT TTT	Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Gly Glu Val Lys Ala Phe	40 45 50				320
GAA GGC AAA GAC CTG GAG GAG CAG TTG CGG TCT GTG TCC AGC GTA GAT	Glu Gly Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp	55 60 65				368
GAG CTG ATG TCT GTC CTG TAC CCA GAC TAC TGG AAA ATG TAC AAG TGC	Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met Tyr Lys Cys	70 75 80				416
CAG CTG CGG AAA GGC GGC TGG CAG CAG CCC ACC CTC AAT ACC AGG ACA	Gln Leu Arg Lys Gly Gly Gln Gln Pro Thr Thr Asn Thr Arg Thr	85 90 95				464
GGG GAC AGT GTA AAA TTT GCT GCT GCA CAT TAT AAC ACA GAG ATC CTG	Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu	100 105 110 115				512
AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGT GAG	Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu	120 125 130				560
GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GCA GCC ACA AAC ACC TTC	Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr Asn Thr Phe	135 140 145				608
TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC AAC	Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn	150 155 160				656
AGC GAG GGG CTG CAG TGC ATG AAC ACC AGC ACA GGT TAC CTC AGC AAG	Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr Leu Ser Lys	165 170 175				704
ACG TTG TTT GAA ATT ACA GTG CCT CTC TCA CAA GGC CCC AAA CCA GTC	Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val	180 185 190 195				752
ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGG TGC ATG TCT AAA CTG	Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu	200 205 210				800
GAT GTT TAC AGA CAA GTT CAT TCA ATT ATT AGA CGT TCT CTG CCA GCA	Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala	215 220 225				848

ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT	896
Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr	
230 235 240	
GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG CAG GAT TTT ATC	944
Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln Asp Phe Ile	
245 250 255	
TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC	992
Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe His Asp Val	
260 265 270 275	
TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC	1040
Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys	
280 285	
AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT	1088
Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Glu Leu Asp	
295 300 305	
AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA	1136
Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Asn Ser	
310 315 320	
TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT	1184
Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys	
325 330 335	
AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC	1232
Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala	
340 345 350 355	
TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG	1280
Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys	
360 365 370	
TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA	1328
Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg	
375 380 385	
CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC	1376
Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg	
390 395 400	
TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCATA	1422
Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn	
405 410 415	
CCAGTTTCA GTCACTCACA GTCATTTACT CTCTTGAAGA CTGTGGAAC AGCACTTAGC	1482
ACTGTCTATG CACAGAAAGA CTCTGTGGGA CCACATGGTA ACAGAGGCCC AAGTCTGTGT	1542
TTATTGAACC ATGTGGATTA CTGCGGAGA GGACTGGCAC TCATGTGCAG AAAAAACCTC	1602
TTCAAGAACT GGTTTTCTGC CAGGGACCAG ACAGCTGAGG TTTTCTCTT GTGATTTAAA	1662
AAAAGAATGA CTATATAATT TATTTCCACT AAAAATATTG TTCTGCAAT CATTTTTATA	1722
GCAATAACAA TTGTTAAAGC TCACTGTGAT CAGTATTTTT ATAACATGCA AAACATATGT	1782
TAAATAAAAA TGAAAAATGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT	1836

EXHIBIT D

Quail VEGF-C

GCCCCCGCCG AGCGCTCCGC GCGCAGCCGC CGGGCCGGGC CGGCCCGGGT	60
GCGAGCGGCC ACTGGGTCTT GCTTCCCTCC TTCTCTCTCC TCTCTCTCTC	120
TCTGGCGTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCGGTTT	180
TTTTTACCTG GCAAAGTCCG GATRACTTCG GTGAGAAITTT GCAAAGAGGC	240
CCTGCAGGCG TCTGGGAGCT GCTGCCGCGC TCGCATCTTC TCCATCCCGC	300
GCCTTGGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAAGAAA	360
GGGGGAGAGA AAAGGAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCCT	420
GCGGCCCCCC TCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC	473
Met His Leu Leu Glu Met Leu	
1. 5	
TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG	521
Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Leu Gly Pro Arg	
10 15 20	
CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG	569
Gln Pro Pro Val Ala Ala Tyr Glu Ser Gly His Gly Tyr Tyr Glu	
25 30 35	
GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG	617
Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu	
40 45 50 55	
GAA GAG CAG TTG CGA TCT GTG TCC AGT GTG GAT GAA CTC ATG ACA GTA	665
Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val	
60 65 70	
CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA	713
Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly	
75 80 85	
GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT	761
Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp	
90 95 100	
GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA	809
Asp Ser Leu Lys Phe Ala Ala His Tyr Asn Ala Glu Ile Leu Lys	
105 110 115	
AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG	857
Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val	
120 125 130 135	
TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT	905
Cys Val Asp Leu Gly Lys Glu Phe Gly Ala Thr Thr Asn Thr Phe Phe	
140 145 150	
AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT	953
Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Gly Cys Cys Asn Ser	
155 160 165	
GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA	1001
Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr	
170 175 180	



TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA	1049
Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr	
185 190 195	
GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT	1097
Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp	
200 205 210 215	
GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA	1145
Val Tyr Arg Gln Val His Ser Ile Ile Arg Ser Leu Pro Ala Thr	
220 225 230	
CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC	1193
Gln Thr Gln Cys His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val	
235 240 245	
TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC	1241
Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe	
250 255 260	
TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT	1289
Ser Ser His Leu Gly Asp Ser Asp Thr Ser Ser Glu Gly Phe His Ile Cys	
265 270 275	
GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA	1337
Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys	
280 285 290 295	
GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG	1385
Gly Gly Val Arg Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg	
300 305 310	
GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT	1433
Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys	
315 320 325	
GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA	1481
Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys	
330 335 340	
AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC	1529
Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys	
345 350 355	
GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT	1577
Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe	
360 365 370 375	
CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCA TGT ACA GTC CGA ACG	1625
His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr	
380 385 390	
AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT	1673
Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys	
395 400 405	
GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT	1726
Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn	
410 415	
CGCTATATAG TGTCG	1741

## EXHIBIT E

## VEGF-C alignment

					50
Hum	1	EMLLGFFSVA	CSLLAAALLP	GPREAPAAAA	AFESGLDLS
Mou		MHLLCFLSLA	CSLLAAALIP	SPREAPATVA	AFESGLGFSE
Qua		MHLEMLSLG	CCLAAGAVLL	GPRQPPVA.A	AYESGHGYE
					EEFGAGEPKA
	51				100
Hum		YASKDLEEQL	RSVSSVDELM	TVLYPEYWK	YKQQLRKGW
Mou		FEKDLLEEQL	RSVSSVDELM	SVLYPDYWK	YKQQLRKGW
Qua		HASKDLEEQL	RSVSSVDELM	TVLYPEYWK	FKQQLRKGW
					QHNREQANLN
					Q....OPTLN
					QHNREHSSSD
	101				150
Hum		SRTEETIKFA	AAHYNTEILK	SIDNEWRTQ	CHPREVCIDV
Mou		TRTGDVVKFA	AAHYNTEILK	SIDNEWRTQ	CHPREVCIDV
Qua		TRSDDSLKFA	AAHYNAEILK	SIDTEWRTQ	GMPREVCIDL
					GKEFGVATNT
					GKEFGAATNT
					GKEFGATTNT
	151				200
Hum		FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTSY	LSKTLFEITV
Mou		FFKPPCVSVY	RCGGCCNSEG	LQCMNTSTGY	LSKTLFEITV
Qua		FFKPPCVSIY	RCGGCCNSEG	LQCMNISTNY	ISKTLFEITV
					PLSQGPKPVT
					PLSQGPKPVT
					PLSHGPKPVT
	201				250
Hum		ISFANHTSCR	CMKSLDVYRQ	VHSIIRSLP	ATLPQCOAAN
Mou		ISFANHTSCR	CMKSLDVYRQ	VHSIIRSLP	ATLPQCOAAN
Qua		VSFANHTSCR	CMKSLDVYRQ	VHSIIRSLP	ATQTCCHVAN
					KTCPTNYMWN
					KTCPTNYVWN
					KTCPKNHVWN
	251				300
Hum		NHICRCLAQE	DFMFSSDAGD	DSTDGFHDIC	GNKELDEET
Mou		NYMCRCLAQQ	DFIFYSNVED	DSTNGFHDVC	GNKELDEET
Qua		NQICRCLAQH	DFGFSSHLGD	SDTSEGFHIC	GNKELDEET
					CQCVCRAGLE
					CQCVCCKGGLR
					CQCVCCKGVR
	301				350
Hum		PASCGPHKEL	DRNSCQCVCCK	NKLFPSQCGA	NREFDENTCQ
Mou		PSSCGPHKEL	DRDSCQCVCCK	NKLFPSNCGA	NREFDENTCQ
Qua		FISCGPHKEL	DRASCQCMCK	NKLLPSSCGP	NKEFDEEKCQ
					CVCKRTCPRN
					CVCKRTCPRN
					CVCKRTCPKH
	351				400
Hum		QPLNPGKAC	ECTESPQRCL	LKGKKFHHQT	CSCYRRPCTN
Mou		QPLNPGKAC	ECTENTQKCF	LKGKKFHHQT	CSCYRRPCAN
Qua		HPLNPAKCIC	ECTESPKNCF	LKGRFHHQT	CSCYRRPCTV
					RTKRCDAAGFL
	401				420
Hum		YSEEVCRCP	SYWKRPMMS*		
Mou		FSEEVCRCP	SYWKRPHLN*		
Qua		LAEEVCRCP	TSWKRPLMN*		

PAGE: 1

RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/585,895A

1812 #21  
DATE: 01/22/98  
TIME: 15:36:47

INPUT SET: S22772.raw

This Raw Listing contains the General  
Information Section and up to the first 5 pages.

SEQUENCE LISTING

ENTERED

(1) General Information:

(i) APPLICANT: Alitalo, Kari  
Joukov, Vladimir

(ii) TITLE OF INVENTION: RECEPTOR LIGAND

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: United States of America  
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 08/585,895  
(B) FILING DATE: 12-JAN-1996  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/510,133  
(B) FILING DATE: 01-AUG-1995

(viii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/340,011  
(B) FILING DATE: 14-NOV-1994

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Cass, David A.  
(B) REGISTRATION NUMBER: 38,153  
(C) REFERENCE/DOCKET NUMBER: 28967/33072

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 312/474-6300  
(B) TELEFAX: 312/474-0448

RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/585,895ADATE: 01/22/98  
TIME: 15:36:49

INPUT SET: S22772.raw

47 (C) TELEX: 25-3856  
48  
49 (2) INFORMATION FOR SEQ ID NO:1:  
50  
51 (i) SEQUENCE CHARACTERISTICS:  
52 (A) LENGTH: 20 base pairs  
53 (B) TYPE: nucleic acid  
54 (C) STRANDEDNESS: single  
55 (D) TOPOLOGY: linear  
56  
57 (ii) MOLECULE TYPE: DNA (genomic)  
58  
59 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
60  
61 TGTCTCGCT GTCCTTCTCT 20  
62  
63 (2) INFORMATION FOR SEQ ID NO:2:  
64  
65 (i) SEQUENCE CHARACTERISTICS:  
66 (A) LENGTH: 70 base pairs  
67 (B) TYPE: nucleic acid  
68 (C) STRANDEDNESS: single  
69 (D) TOPOLOGY: linear  
70  
71 (ii) MOLECULE TYPE: DNA (genomic)  
72  
73 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
74  
75 ACATGCATGC CACCATGCAG CGGGCCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG 60  
76  
77 GACTCCTGGA 70  
78  
79 (2) INFORMATION FOR SEQ ID NO:3:  
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81 (i) SEQUENCE CHARACTERISTICS:  
82 (A) LENGTH: 24 base pairs  
83 (B) TYPE: nucleic acid  
84 (C) STRANDEDNESS: single  
85 (D) TOPOLOGY: linear  
86  
87 (ii) MOLECULE TYPE: DNA (genomic)  
88  
89 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
90  
91 ACATGCATGC CCCGCCGTC ATCC 24  
92  
93 (2) INFORMATION FOR SEQ ID NO:4:  
94  
95 (i) SEQUENCE CHARACTERISTICS:  
96 (A) LENGTH: 22 base pairs  
97 (B) TYPE: nucleic acid  
98 (C) STRANDEDNESS: single  
99 (D) TOPOLOGY: linear

INPUT SET: S22772.raw

100 (11) MOLECULE TYPE: DNA (genomic)  
101  
102 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
103  
104 CGGAATTCCC CATGACCCCA AC 22  
105  
106 (2) INFORMATION FOR SEQ ID NO:5:  
107  
108 (1) SEQUENCE CHARACTERISTICS:  
109 (A) LENGTH: 33 base pairs  
110 (B) TYPE: nucleic acid  
111 (C) STRANDEDNESS: single  
112 (D) TOPOLOGY: linear  
113  
114 (11) MOLECULE TYPE: DNA (genomic)  
115  
116 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  
117  
118 CCATCGATGG ATCTACCTG AAGCCGCTT CTT 33  
119  
120 (2) INFORMATION FOR SEQ ID NO:6:  
121  
122 (1) SEQUENCE CHARACTERISTICS:  
123 (A) LENGTH: 17 base pairs  
124 (B) TYPE: nucleic acid  
125 (C) STRANDEDNESS: single  
126 (D) TOPOLOGY: linear  
127  
128 (11) MOLECULE TYPE: DNA (genomic)  
129  
130 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:  
131  
132 ATTTAGGTGA CACTATA 17  
133  
134 (2) INFORMATION FOR SEQ ID NO:7:  
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136 (1) SEQUENCE CHARACTERISTICS:  
137 (A) LENGTH: 34 base pairs  
138 (B) TYPE: nucleic acid  
139 (C) STRANDEDNESS: single  
140 (D) TOPOLOGY: linear  
141  
142 (11) MOLECULE TYPE: DNA (genomic)  
143  
144 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:  
145  
146 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34  
147  
148 (2) INFORMATION FOR SEQ ID NO:8:  
149  
150 (1) SEQUENCE CHARACTERISTICS:  
151 (A) LENGTH: 40 amino acids  
152

PAGE: 4

RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/585,895A

DATE: 01/22/99  
TIME: 15:36:54

INPUT SET: S22772.raw

153 (B) TYPE: amino acid  
154 (C) STRANDEDNESS: single  
155 (D) TOPOLOGY: linear  
156  
157 (ii) MOLECULE TYPE: protein  
158  
159 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:  
160  
161 Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp  
162 1 5 10 15  
163  
164 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg  
165 20 25 30  
166  
167 His Arg Gln Glu Ser Gly Phe Arg  
168 35 40  
169  
170 (2) INFORMATION FOR SEQ ID NO:9:  
171  
172 (i) SEQUENCE CHARACTERISTICS:  
173 (A) LENGTH: 21 base pairs  
174 (B) TYPE: nucleic acid  
175 (C) STRANDEDNESS: single  
176 (D) TOPOLOGY: linear  
177  
178 (ii) MOLECULE TYPE: DNA (genomic)  
179  
180 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:  
181  
182 CTGGAGTCGA CTGGCGGAC T 21  
183  
184 (2) INFORMATION FOR SEQ ID NO:10:  
185  
186 (i) SEQUENCE CHARACTERISTICS:  
187 (A) LENGTH: 60 base pairs  
188 (B) TYPE: nucleic acid  
189 (C) STRANDEDNESS: single  
190 (D) TOPOLOGY: linear  
191  
192 (ii) MOLECULE TYPE: DNA (genomic)  
193  
194 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
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196 CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC 60  
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198 (2) INFORMATION FOR SEQ ID NO:11:  
199  
200 (i) SEQUENCE CHARACTERISTICS:  
201 (A) LENGTH: 34 base pairs  
202 (B) TYPE: nucleic acid  
203 (C) STRANDEDNESS: single  
204 (D) TOPOLOGY: linear  
205

PAGE: 5

RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98  
TIME: 15:36:56

INPUT SET: S22772.raw

```
206      (ii) MOLECULE TYPE: DNA (genomic)
207
208      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
209
210      CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC
211
212      (2) INFORMATION FOR SEQ ID NO:12:
213
214      (i) SEQUENCE CHARACTERISTICS:
215          (A) LENGTH: 20 base pairs
216          (B) TYPE: nucleic acid
217          (C) STRANDEDNESS: single
218          (D) TOPOLOGY: linear
219
220      (ii) MOLECULE TYPE: DNA (genomic)
221
222      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
223
224      GTTGCCCTGTG ATGTGCACCA
225
226      (2) INFORMATION FOR SEQ ID NO:13:
227
228      (i) SEQUENCE CHARACTERISTICS:
229          (A) LENGTH: 18 amino acids
230          (B) TYPE: amino acid
231          (C) STRANDEDNESS: single
232          (D) TOPOLOGY: linear
233
234      (ii) MOLECULE TYPE: peptide
235
236      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
237
238      Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
239      1             5             10             15
240
241      Leu Lys
242
243
244      (2) INFORMATION FOR SEQ ID NO:14:
245
246      (i) SEQUENCE CHARACTERISTICS:
247          (A) LENGTH: 17 base pairs
248          (B) TYPE: nucleic acid
249          (C) STRANDEDNESS: single
250          (D) TOPOLOGY: linear
251
252      (ii) MOLECULE TYPE: DNA (genomic)
253
254      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
255
256      CCAGARGARA CNATHAA
257
258      (2) INFORMATION FOR SEQ ID NO:15:
```

17

PAGE: 1

SEQUENCE VERIFICATION REPORT  
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98  
TIME: 15:36:59

INPUT SET: S22772.raw

Line

Error

Original Text





**PATENT**  
**28967/33072**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:	)	Title: RECEPTOR LIGAND
Alitalo et al.	)	
Serial No. 08/585,895	)	Art Unit: 1801
Filed: January 12, 1996	)	Examiner: Lathrop, B.

**Change of Inventor's Address**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please be advised that the residence and mailing address of co-inventor Vladimir Joukov is now as follows:

51 Massachusetts Avenue, Apt. 1F  
Boston, Massachusetts 02115

This notification is NOT intended as a change of correspondence address. Please continue to send correspondence to the Applicants' attorney at the address below:

Respectfully submitted,  
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: David A. Gass

David A. Gass  
Registration No. 38,153

Date: Feb 24, 1998

## ASSIGNMENT

WHEREAS Helsinki University Licensing, Ltd., Viikinkaari 8 A, FIN-00710 Helsinki, Finland (hereinafter HUL), its successors and assigns, is the assignee of the entire right, title and interest in the invention or improvements of Kari Alitalo and Vladimir Joukov relating to the cloning, isolation and sequencing of human Vascular Endothelial Growth Factor C (VEGF-C) disclosed in certain applications for Letters Patent of the United States, and in said applications and any and all other applications, both United States and foreign, which Kari Alitalo and Vladimir Joukov may file, either solely or jointly with others, on said invention or improvements, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said applications, and in any reissue or extension thereof; and

WHEREAS, for ten dollars (\$10.00), and other good and valuable consideration enumerated in a written agreement dated 24 October 1996, the sufficiency of which is hereby acknowledged, HUL has agreed to share ownership of the aforementioned invention, improvements, applications, patents, reissues, extensions, and the like on a 50% / 50% equal basis with Ludwig Institute for Cancer Research, a Swiss not-for-profit corporation having an office at 1345 Avenue of the Americas, New York, New York 10105, United States of America (hereinafter LICR);

NOW, THEREFORE, HUL hereby assigns to LICR a fifty percent (50%) interest in the patent applications identified in the following LIST OF PATENT PROPERTIES, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said patent applications, and in any reissue or extension thereof.

### LIST OF PATENT PROPERTIES

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>
08/510,133	01/08/95	Receptor Ligand
08/585,895	12/01/96	Receptor Ligand
08/601,132	14/02/96	Receptor Ligand
08/671,573	28/06/96	Receptor Ligand VEGF-C
PCT/FI96/00427	01/08/96	Receptor Ligand VEGF-C
08/795,430	02/05/97	Vascular Endothelial Growth Factor C (VEGF-C) Protein and Gene, Mutants Thereof, and Uses Thereof

WITNESS my hand this 25 day of April, Nineteen Hundred and Ninety-Seven.

Witnesses:

1) [Signature]  
Name:

2) [Signature]  
Name:

Helsinki University  
Licensing Ltd.

By: [Signature]  
Heikki Lampi  
President



## POWER OF ATTORNEY

The Ludwig Institute for Cancer Research hereby  
appoints:

Alvin D. Shulman (19,412)  
Owen J. Murray (22,111)  
Allen H. Gerstein (22,218)  
Nate F. Scarpelli (22,320)  
Edward M. O'Toole (22,477)  
Michael F. Borun (25,447)  
Trevor B. Jolke (25,342)

Timothy J. Vezau (26,348)  
Carl E. Moore, Jr. (26,487)  
Richard H. Anderson (26,526)  
Patrick D. Ertel (26,877)  
James P. Zoller (28,491)  
William E. McCracken (30,195)  
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Richard A. Schnurr (30,890)  
Anthony Nimmo (30,920)  
Christine A. Dudzik (31,245)  
Kevin D. Hogg (31,839)  
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Martin J. Hirsch (32,237)

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Karl A. Vick (33,288)  
Douglas C. Hochstetler (33,710)  
Cynthia L. Schaller (34,245)  
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and  
revocation, to act on its behalf before the U.S. Patent and  
Trademark Office in connection with the following applications  
filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Rec'd &amp; Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
United States of America  
(312) 474-6300

Ludwig Institute for Cancer Research  
1345 Avenue of the Americas  
New York, New York 10105

(Date) 26-01-98

By: 

Name: A. MUNRO NTULI

Title: ASSOCIATE DIRECTOR



## POWER OF ATTORNEY

Helsinki University Licensing, Ltd., hereby  
appoints:

Alvin D. Shulman (19,412)  
Owen J. Murray (22,111)  
Allen H. Gerstein (22,218)  
Nate F. Scarpelli (22,320)  
Edward M. O'Toole (22,477)  
Michael F. Borun (25,447)  
Trevor B. Joike (25,542)

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Richard H. Anderson (26,526)  
Patrick D. Ertel (26,877)  
James P. Zeller (28,491)  
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Jeffrey S. Sharp (31,879)  
Martin J. Hirsch (32,237)

James J. Napoli (32,361)  
Richard M. Le Bargo (32,254)  
Karl A. Vick (33,288)  
Douglas C. Hochstetler (33,710)  
Cynthia L. Schaller (34,245)  
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and  
revocation, to act on its behalf before the U.S. Patent and  
Trademark Office in connection with the following applications  
filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Reel &amp; Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 Receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
United States of America  
(312) 474-6300

Helsinki University Licensing, Ltd.  
Viikinkaari 8 A  
FIN-00710 Helsinki  
FINLAND

(Date)

28th of June 1998

By:

Name: Heikki Lampi

Title: President

Please enter the power of attorney documents into the file for the above-identified patent application.

Respectfully submitted,  
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By:



David A. Gass  
Registration No. 38,153

Date: Feb 24, 1998



G-AU-1801  
1652

PATENT #21  
28967/33072  
02/12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	I hereby certify that this paper is
	)	being deposited with the United
Alitalo et al.	)	States Postal Service as first class
	)	mail, postage prepaid, in an
Serial No. 08/585,895	)	envelope addressed to: Assistant
	)	Commissioner for Patents,
Filed: January 12, 1996	)	Washington, D.C. 20231, on this
	)	date:
For: RECEPTOR LIGAND	)	Dated: <u>Feb 24, 1998</u>
	)	
Art Unit: 1801	)	<u>David A. Gass</u>
	)	David A. Gass
Examiner: Lathrop, B.	)	

TRANSMITTAL OF POWERS OF ATTORNEY

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith are power of attorney documents executed by the two assignees of the above-identified patent application: Helsinki University Licensing, Ltd., and The Ludwig Institute for Cancer Research.

The above-identified application was assigned by the inventors to Helsinki University Licensing, Ltd., (HUL) in an assignment recorded at Reel 8145, Frame 0829.

HUL assigned a 50% interest in the application to The Ludwig Institute for Cancer Research, as evidenced by the attached assignment document which has been submitted for recordation.



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
-----------------	-------------	----------------------	---------------------

08/585,895 01/12/96 ALITALO

K 28113/33072

EXAMINER

HM11/0324

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

BROWN, K

ART UNIT PAPER NUMBER

1646

DATE MAILED:

03/24/98

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

# Office Action Summary

Application No. 08/585,895 Applicant(s) Alitalo et al.  
 Examiner Brown Group Art Unit 1646

—The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address—

## Period for Response

A SHORTENED STATUTORY PERIOD FOR RESPONSE IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a response be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for response specified above is less than thirty (30) days, a response within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for response is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to respond within the set or extended period for response will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

## Status

- ☒ Responsive to communication(s) filed on 12/1/97
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

## Disposition of Claims

- ☒ Claim(s) 1, 3-5, 7, 11, 18-37 is/are pending in the application.
- ☐ Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- ☐ Claim(s) 37-36 is/are allowed.
- ☒ Claim(s) 1, 3-5, 7, 11, 18-32, 37-38 is/are rejected.
- ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- ☐ Claim(s) \_\_\_\_\_ are subject to restriction or election requirement.

## Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.
- ☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

## Priority under 35 U.S.C. § 119 (a)-(d)

- ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
  - ☐ All ☐ Some ☐ None of the CERTIFIED copies of the priority documents have been received.
  - ☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_
  - ☐ received in this national stage application from the International Bureau (PCT Rule 1.7.2(a)).

\*Certified copies not received: \_\_\_\_\_

## Attachment(s)

- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). \_\_\_\_\_ ☐ Interview Summary, PTO-413
- ☒ Notice of References Cited, PTO-892 ☐ Notice of Informal Patent Application, PTO-152
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948 ☐ Other \_\_\_\_\_

Office Action Summary



Serial Number: 08/585,895

Page 2

Art Unit: 1646

#### DETAILED ACTION

1. The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1646.

#### *Response to Amendment*

2. Claims 2, 6, 8-10 and 12-17 have been cancelled, and claims 26-38 have been added. Therefore, claims 1, 3-5, 7, 11 and 18-38 are instantly examined.

3. The following rejections are withdrawn upon reconsideration and Applicant's amendments: The rejection of claims 1 and 18-25 under 35 USC 112, first paragraph, and the rejection of claims 3-5, 11 and 18-25 under 35 USC 112, second paragraph.

4. The declaration under 37 CFR 1.132 filed 1 December 1997 is insufficient to overcome the rejection of claims 7 and 37 based upon 35 USC 112, first paragraph, as set forth in this Office action in ¶10 below for the following reasons: Although the declaration of Alitalo at ¶5 states that complete sequencing of the cDNA insert contains the sequence of SEQ ID NO:44, the declaration does not state that the cDNA insert is derived from ATCC Deposit No. 97231. The declaration also does not state what is the relationship of the 1997 base pair cDNA to the "approximately 2.1 kb insert" of the pFLT4-L clone. If the "approximately 2.1 kb insert" of ATCC Deposit No. 97231 is what was sequenced and shown to be 1997 base pairs and have the sequence of SEQ ID NO:44, then this should be made clear. In addition, Applicant must state or declare that all restrictions regarding the availability of the deposited material must be irrevocably

Art Unit: 1646

removed upon the granting of the patent (see Paper No. 17, page 4, and below in ¶9-11 of this Office action).

5. The declaration is also insufficient to obviate the rejection of claims 1, 18, 23-31, 37 and 38 under 35 USC 112, first paragraph, as set forth in ¶12 of this Office action for the following reasons: Although the declaration of Alitalo demonstrates at ¶7-18 that fragments comprising portions of SEQ ID NO:33 bind to the Flt4 receptor and activate tyrosine phosphorylation, and that polynucleotides which hybridize to SEQ ID NO:32 encode VEGF-C polypeptides which bind to the Flt4 receptor and activate tyrosine phosphorylation, the showing is not commensurate in scope with the claims, which recite any protein which binds to Flt4 receptor, for those reasons provided in ¶12 of this Office action.

*Oath/Declaration*

6. The requirement for a new declaration is withdrawn in view of Applicant's second declaration, filed 12 August 1996.

*Drawings*

7. The proposed drawing correction and/or the proposed substitute sheets of drawings, filed on 1 December 1997 have been approved.

8. The wish to defer formal corrections of the drawings and the petition for photographs is acknowledged.

Art Unit: 1646

*Sequence Rules*

9. The submission of a new Sequence Listing and CRF containing the sequences of Figures 9B and 10 is acknowledged, and the requirement to comply with Sequence Rules is withdrawn.

*Specification*

10. The amendment filed 1 December 1997 is objected to under 35 U.S.C. 132 because it introduces new matter into the disclosure. 35 U.S.C. 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: SEQ ID NOS: 44 and 45.

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO:44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO:44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L, was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO:44." It is further noted that the nucleotide sequence of the plasmid is not SEQ ID NO:45, as stated in the added sentence. SEQ ID NO:45 is a translated open reading frame of the nucleotide sequence of SEQ ID NO:44, as stated by Dr. Kari Alitalo (¶5). In addition, Applicant states that ATCC Deposit No. 97231 has been deposited under the terms of the Budapest Treaty; however,

Serial Number: 08/585,895

Page 5

Art Unit: 1646

Applicant must also state that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent (37 CFR 1.808), as discussed in Paper No. 17, page 4. If this statement is made, the objection to the specification will be withdrawn.

*Claim Rejections - 35 USC § 112*

11. Claims 7 and 37 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This rejection is maintained for reasons of record set forth in Paper No. 17, pages 4-5.

12. Applicant's arguments filed 1 December 1997 have been fully considered but they are not persuasive.

Applicant argues that ATCC Deposit No. 97231 has been deposited under the terms of the Budapest Treaty; however, Applicant must also state that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent (37 CFR 1.808), as discussed in Paper No. 17, page 4. If this statement is made, the rejection will be withdrawn.

13. Claims 1, 18, 23-31, 37 and 38 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for while being enabling for a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 sufficient to bind to the Flt4

Art Unit: 1646

receptor tyrosine kinase and stimulate tyrosine phosphorylation of the Flt4 receptor, does not reasonably provide enablement for a polynucleotide which encodes polypeptide which is defined only by its binding to the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Claims 1, 18, 23-31, 37 and 38 are not commensurate in scope with the specification with respect to the recitation in claims 1, 26-29 and 37 of a polynucleotide which hybridizes to SEQ ID NO:32 and which encodes a polypeptide which binds to the Flt4 receptor or in claims 18, 23-25, 30-31 and 38 of a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 which binds to the Flt4 receptor. One skilled in the art could use the guidance in the specification to isolate polynucleotides which hybridize to SEQ ID NO:32 and test them for Flt4 receptor binding and tyrosine phosphorylation activity. Similarly, one skilled in the art could use the guidance in the specification to isolate polynucleotide which encode polypeptides which comprise portions of SEQ ID NOS:33 and test them for Flt4 receptor binding and tyrosine phosphorylation activity. However, claims 1, 18, 23-31, 37 and 38 currently recite only a polynucleotide which encodes a polypeptide which binds the Flt4 receptor, and thus these claims encompass polypeptides which bind to the Flt4 receptor under any condition and which have no biological activity. It is well-known in the art that a growth factor ligand must not only bind to its receptor but also be able to induce some biochemical signal, such as phosphorylation, in order to have a biological effect (Borg, p. 981, col. 2). Neither the specification nor the prior art teaches

Serial Number: 08/585,895

Page 7

Art Unit: 1646

one skilled in the art how to use a polypeptide which binds to the Flt4 receptor and which does not stimulate the tyrosine phosphorylation activity of the receptor. Furthermore, given the large number of different biochemical pathways which may or may not be activated by polypeptide binding, and given the lack of guidance in the specification, one skilled in the art would not know which of the many biochemical signaling pathways, other than tyrosine phosphorylation, to examine in order to determine whether a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 might activate. Similarly, one skilled in the art would not know how to use a polynucleotide which hybridized to SEQ ID NO:32 and which encoded a polypeptide which bound to Flt4 receptor but which did not activate tyrosine phosphorylation. Absent such guidance, one skilled in the art would not know how to use a polynucleotide which encoded a polypeptide which binds to the Flt4 receptor but which does not stimulate tyrosine phosphorylation. Therefore, it would require undue experimentation to practice this invention as claimed.

This rejection could be overcome by amending the claims to recite that the encoded polypeptide not only binds to the human Flt4 receptor but stimulates tyrosine phosphorylation of the Flt4 receptor tyrosine kinase, such as is recited in claim 19.

14. Applicant's arguments regarding the previous rejection under 35 USC 112, first paragraph, have not been addressed as the previous rejection of the claims has been withdrawn.

Art Unit: 1646

15. Claims 1, 3-5, 7, 11, 18-30, 32 and 37-38 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

16. Claims 1, 3-5, 7, 26-29 and 37 are indefinite with respect to the term "a domain defined by eight conserved cysteine residues." It is unclear to what the eight residues are conserved. It is also unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter. Furthermore, these claims are indefinite with respect to the term "having homology to vascular endothelial growth factor." It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF (see Reeck et al. Cell, 50, 667).

17. Claims 1, 3-5, 7, 11, 18-30, 32 and 37-38 are indefinite because it is unclear what is a domain encompassed by "cysteine motifs of a Balbiani ring 3 protein." Since the BR3P domain is not defined in the specification, one cannot determine what a BR3P domain is. Furthermore, it is unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter.

18. Claims 1, 26-29 and 37 are indefinite with respect to the term "high affinity." The term "high affinity" is relative, and it is not clear how strongly a protein must bind to the Flt4 receptor in order for it to be considered "high affinity." It is suggested that the claims be amended to recite a particular range of  $K_d$ .

Serial Number: 08/585,895

Page 9

Art Unit: 1646

19. Claims 1, 3-5, 7, 26-30 and 37 are indefinite with respect to the term "including." It is unclear whether "including" is equivalent to the open language "comprising" or to the closed language "consisting of."
20. Claims 3, 5, 18, 24-25 and 30-31 are indefinite because the term "said polynucleotide" lacks antecedent basis.
21. Claim 30 is indefinite with respect to the term "VEGF-homologous portion." It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF (see Reeck et al. Cell, 50, 667).
22. Claim 32 is indefinite with respect to an amino acid sequence "corresponding to" another amino acid sequence. It is unclear whether "corresponding to" means that the amino acid sequence is identical or not.
23. Applicant's arguments regarding the previous rejection under 35 USC 112, second paragraph, have not been addressed as the previous rejection of the claims has been withdrawn.
24. Applicant is correct that the publication date of Reference B1 does not antedate the effective filing date of the instant application, and thus does not anticipate or render obvious the claimed invention because it is not available as prior art.
25. Applicant's arguments regarding Reference B1 are noted; however, since no rejection has been made over this patent, these arguments are not addressed.

*Conclusion*

26. Claims 33-36 are allowed.



Serial Number: 08/585,895

Page 10

Art Unit: 1646

27. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Brown whose telephone number is (703) 308-3667. The examiner can normally be reached on Mondays through Thursdays and on alternate Fridays from 8:30 to 6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Stephen Walsh, can be reached on (703) 308-2957.

Official papers filed by fax should be directed to (703) 305-4242. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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20 March 1998

*Stephen Walsh*  
STEPHEN WALSH  
SUPERVISORY PATENT EXAMINER  
GROUP 1800

<b>Notice of References Cited</b>				Application No. <b>081535395</b>		Applicant(s) <b>Alitelo et al.</b>	
				Examiner <b>Brown</b>		Group Art Unit <b>1646</b>	
						Page <b>L</b> of <b>1</b>	
U.S. PATENT DOCUMENTS							
*	DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS		
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NON-PATENT DOCUMENTS							
*	DOCUMENT (Including Author, Title, Source, and Pertinent Pages)						DATE
U	<b>Reuck et al. Cell 50,667.</b>						<b>1987.</b>
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14. Information Disclosure Statement  
3/21/97
15. Information Disclosure Statement  
4/16/97
16. Office Action  
5/25/97
17. Amendment and Reply  
11/26/97
18. Transmittal of Powers of Attorney/Change of Inventors Address  
2/24/98
19. Office Action  
3/24/98
20. Amendment and Reply  
7/23/98
21. Office Action  
10/8/98
22. Supplemental Information Disclosure Statement  
7/26/99
23. Supplemental Information Disclosure Statement  
10/26/99
24. Office Action  
4/4/00
25. Associate Power of Attorney  
6/22/00
26. Office Action  
6/29/00
27. Amendment and Reply  
8/4/00
28. Amendment After Allowance/Request for Approval of Drawing Changes  
1/24/01

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29. Issue Fee Transmittal  
1/24/01

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